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(54) Title: HYPOGLYCEMIC AGENT FROM CRYPTOLEPIS		
(57) Abstract		
The use of extracts from <i>Cryptolepis sp.</i> or quindoline alkaloids such as quindoline and cryptolepine contained therein as hypoglycemic agents, as well as methods for obtaining the hypoglycemic agents are described. According to a preferred embodiment, the extracts are derived from <i>Cryptolepis sanguinolenta</i> . As hypoglycemic agents, the extracts or quindoline alkaloids such as quindoline or cryptolepine are useful for treating insulin-dependent (type I) and non-insulin-dependent (type II) diabetes.	Isolation of Cryptolepine from <i>Cryptolepis sanguinolenta</i> roots	

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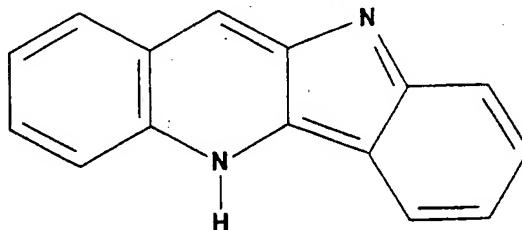
HYPOLYCEMIC AGENT FROM CRYPTOLEPIS1. FIELD OF THE INVENTION

This invention pertains to the use of a hypoglycemic agent from *Cryptolepis* sp., including, but not limited to *C. triangularis* and *C. sanguinolenta*, and quindoline alkaloids contained therein, such as quindoline and cryptolepine, for the treatment of diabetes mellitus.

10 2. BACKGROUND OF THE INVENTION

Compounds of the quindoline family of alkaloids, e.g. quindoline:

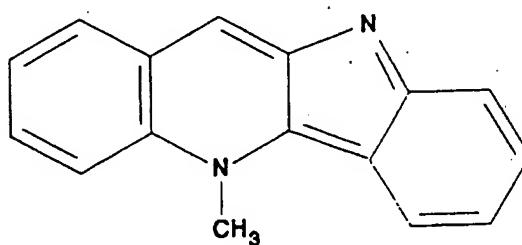
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and cryptolepine:

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were first synthesized in 1906 (Fichter, F. and Boehringer, 35 R., *Chemische Berichte* 39, 3932 (1906)).
The natural product cryptolepine was first isolated from the plant *Cryptolepis triangularis* of the family

P riplocaceae in 1929 (Clinquart, E., Bull. Acad. R. Med. Belg. 12, 6237 (1929)). Cryptolepine was reisolated from *Cryptolepis sanguinolenta* in 1951 (Gellert, E., Raymond-Hamet, Schlittler, E., Helv. Chim. Acta 34, 642 (1951)) and again in 5 1978 together with quindoline and other uncharacterized alkaloids (Dwuma-Badu, D., Ayim, J.S.K., Fiagbe, N.I.Y., Knapp, J.E., Schiff Jr., P.L., and Slatkin, D.J., J. Pharm. Sci. 67, 433 (1978)). In a study of Sri-Lankan medicinal plants, cryptolepine was identified as the major alkaloid in 10 *Sida acuta* (Gunatilaka, A.A., Planta Medica 39, 66 (1980)).

Proton and carbon n.m.r. (DMSO₆) spectra for cryptolepine were assigned by Tackie, A.N., Sharaf, M.H.M., Schiff Jr., P.L., Boye, G.L., Couch, R.C., and Martin, G.E. (J. Heterocycl. Chem. 28, 1429 (1991)); and by Ablordeppy, S.Y; 15 Hufford, C.D.; Borne, R.F., and Dwuma-Badu, D. Planta. Medica. 56, 416 (1990).

A study conducted in 1937 described marked hypothermia in dogs after administration of cryptolepine and noted an antagonistic effect to epinephrine characterized by 20 decreased hypertension and renal vasoconstriction; a lethal dose was determined in guinea pigs after i.p. injection as 120 mg/kg (Raymond-Hamet, C., C.R. Soc. Biol. 126, 768 (1937)). A 1938 study showed a marked and protracted hypotensive response in vasectomized dogs after i.v. administration of cryptolepine 25 (Raymond-Hamet, C., C.R. Acad. Sci., 208, 105 (1938)).

A report claimed that cryptolepine possessed hypotensive and antibacterial properties (Drugs Future, 7(7), 466 (1982)). More detailed antimicrobial activities for 30 alcoholic extracts of *Cryptolepis sanguinolenta* were described (Boakye-Yiadom, K. and Heman-Ackah, S.M., J. Pharm. Sci. 68, 1510 (1979)).

In 1980, a paper described the noradrenoreceptor antagonism of cryptolepine in isolated rat *vas deferens* (Noamesi, B.K. and Bangbose, S.O.A., Planta Medica 39, 51 35 (1980)). Cryptolepine's antiinflammatory action was reported in 1981 by the same researchers who identified prostaglandin

E2 antagonism as the cause. (Bambose, S.O.A. and Noamesi, B.K., *Planta Medica*, 41, 392 (1981)).

According to the West African Pharmaceutical Federation *Cryptolepis sanguinolenta* has been widely used in 5 folk medicine for the treatment of malaria, gonorrhoea, and hypertension. The antimalarial activity of *Cryptolepis* roots is linked to the presence of cryptolepine. As an antiinflammatory agent it is claimed to be as active as piroxicam or indomethacin (IMS Pharmaceutical Marketletter, 10 May 7, 1984, p. 13.)

The effects of cryptolepine alone and in combination with dipyridamole were studied in a mouse model of arterial thrombosis in 1989 (Oyekan, A. O. and Okafor, J.P.O., J. Ethnopharmacology 27, 141 (1989)).

15 *Cryptolepine* was found to possess high anti-*Candida* activity as well as pronounced activity against Gram-positive bacteria. Additionally, weak activity against some Gram-negative bacteria but no antiviral activity was observed (Cimanga, K., Pieters, L., Clayes, M., Vanden Berghe, D., and 20 Vlientick, A.J., *Planta Medica* 57, Supp. 2, A98, (1991)).

More recently, cryptolepine and related alkaloids were found to possess antimuscarinic activity at the M1, M2, and M3 receptors (Rauwald, H.W., Kober, N.C., Mutschler, E., and Lambrecht, G., *Planta Medica*, 58, 486 (1992)).

25 *Cryptolepine* has been described to have an effect on collagen-induced platelet aggregation and on the mobilization and metabolism of arachidonic acid in a rabbit model (Oyekan, A.O. and Ablordeppay, S.Y., *Gen. Pharmacol.* 24, 1285 (1993)). *Cryptolepine*'s hypotensive and vasodilator mechanisms of 30 action on perfused rat kidney were further described (Oyekan, A.O., *J. Cardiovasc. Pharmacol.*, 23, 602 (1994)).

Citation or identification of any reference in Section 2 of this application shall not be construed as an admission of prior art to the present invention.

35 To the knowledge of the inventors, no prior study has described any hypoglycemic activity of extracts of *Cryptolepis* sp. nor was there any suggestion in the prior art

that quindoline alkaloids such as cryptolepine or quindoline would be useful as hypoglycemic agents.

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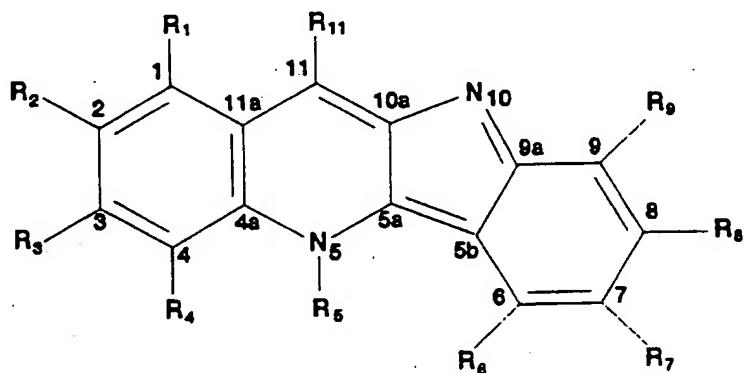
3. SUMMARY OF THE INVENTION

The present invention provides a method for the use of extracts from *Cryptolepis* sp. and compounds of the quindoline family of alkaloids such as quindoline, cryptolepine, etc., as well as pharmaceutically acceptable salts thereof, as hypoglycemic agents or as agents to lower triglyceride levels, particularly in diabetic subjects.

Thus, the invention encompasses a method for using a compound having the formula:

15

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said compound selected from group consisting of:

- (a) where $R_1 - R_{11} = H$;
- (b) where $R_1 - R_4$ and $R_6 - R_{11} = H$, and $R_5 = CH_3$;
- (c) where $R_1 - R_4$ and $R_6 - R_{11} = H$, and R_5 is selected from the group of ethyl, isopropyl, benzyl, 4-hydroxybenzyl, 4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl, 3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl, 3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, and 4-methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl,

4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino,
2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-
dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl,
3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-
5 chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-
fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-
iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-
pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-
bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;

10 (d) where R_1 , R_4 and $R_6 - R_{11} = H$, $R_2 = R_3 =$
- CH_2-O-CH_2- , and R_5 is selected from the group consisting of
ethyl, isopropyl, benzyl, 4-hydroxybenzyl,
4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl,
3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl,
15 3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-
hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-
dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-
methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl,
4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino,
20 2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-
dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl,
3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-
chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-
fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-
25 iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-
pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-
bromophenyl, 4-fluorophenyl, cyclopropyl and isobutyl;

(e) where $R_1 - R_4$, and $R_6 - R_7$, and $R_{10} - R_{11} = H$, $R_8 =$
 $R_9 = -CH_2-O-CH_2-$, and R_5 is selected from the group consisting
30 of ethyl, isopropyl, benzyl, 4-hydroxybenzyl,
4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl,
3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl,
3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-
hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-
35 dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-
methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl,
4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino,

2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl and isobutyl;

5 (f) where $R_1 - R_3$ and $R_6 - R_{11} = H$, and $R_4 - R_5 =$

10 $-\text{CH}_2\text{CH}_2-$;

(g) where $R_1 - R_3$ and $R_6 - R_{11} = H$, and $R_4 - R_5 =$
 $-\text{CH}_2\text{CH}_2\text{CH}_2-$;

(h) where $R_1 - R_4$ and $R_6 - R_{11} = H$, $R_5 = \text{CH}_3$, and
10a, 11 = dihydro;

15 (i) where $R_1 - R_4$ and $R_6 - R_{11} = H$, $R_5 = \text{CH}_3$, and 5a,
5b = dihydro;

(j) where $R_1 - R_4$ and $R_6 - R_{11} = H$, $R_5 = \text{CH}_3$, and 5a,
5b, 10a, 11 = tetrahydro.

(k) where $R_1 - R_{11} = H$, and 9a, 10 = dihydro; and

20 (l) where $R_1 - R_{11} = H$, and 10-methyl as a
hypoglycemic agent.

By "10a, 11 = dihydro" is meant those compounds which bear hydrogen atoms at carbons 10 and 11. By "5a, 5b = dihydro" is meant those compounds which bear hydrogen atoms at carbons 5a and 5b. By "5a, 5b, 10a, 11 = tetrahydro" is meant those compounds which bear hydrogen atoms at carbons 5a, 5b, 10a and 11. By "10-methyl" is meant those compounds which bear a methyl group on N_{10} . In the latter case, the hydrogen atom at R_5 may be optionally absent.

30 The invention also encompasses a method for using a compound of formula I as a triglyceride lowering agent in diabetic subjects. The invention also encompasses novel compounds of formula I as well as pharmaceutical compositions comprising such compounds as well as methods for using such 35 compounds and pharmaceutically acceptable salts thereof.

The invention further encompasses pharmaceutical compositions and methods for using the extracts, or quindoline alkaloids such as quindoline or cryptolepine or pharmaceutically acceptable salts, for the treatment of 5 insulin-dependent diabetes mellitus and non-insulin dependent diabetes mellitus in mammals, including humans. Also encompassed are methods for using the hypoglycemic agents to reduce blood glucose in mammals, including humans, in situations of acute stress.

10 According to a first embodiment of the invention, a therapeutically effective hypoglycemic agent is prepared by a method comprising:

(a) washing a root of *Cryptolepis* sp. with an alcoholic solvent to obtain an alkaloid salt-containing 15 alcoholic solution; and

(b) concentrating the alkaloid salt-containing alcoholic solution to obtain an alkaloid salt-containing residue which is useful as a hypoglycemic agent. According to one mode of the embodiment, the hypoglycemic agent is prepared 20 by said method, further comprising:

(c) diluting the alkaloid salt-containing residue with water to obtain an alkaloid salt-containing aqueous solution;

(d) washing the alkaloid salt-containing aqueous 25 solution with a non-polar organic solvent to remove neutral extractables therefrom;

(e) washing the alkaloid salt-containing aqueous solution of step (d) with base to obtain a free base-containing aqueous solution;

30 (f) extracting the free base-containing aqueous solution with a non-polar organic solvent to obtain a free base-containing organic solution;

(g) concentrating the free base-containing organic solution to obtain a free base-containing residue; and

35 (h) purifying the free base-containing residue to obtain a hypoglycemic agent.

According to a second embodiment of the invention, a therapeutically effective hypoglycemic agent is prepared by a method comprising:

- (a) washing a root of *Cryptolepis* sp. with an organic solvent to obtain an alkaloid salt-containing marc, said alkaloid salt-containing marc being free of neutral extractables;
- (b) wetting the alkaloid salt-containing marc with base to obtain a free base-containing marc;
- 10 (c) washing the free base-containing marc with an alcoholic solvent to obtain a free base-containing alcoholic solution;
- (d) concentrating the free base-containing alcoholic solution to obtain a free base-containing residue;
- 15 and
- (e) purifying the free base-containing residue to obtain a hypoglycemic agent.

Other embodiments of the present invention include washing a root of *Cryptolepis* sp. with a polar alcoholic solvent, an acidic solution or water to obtain an extract, 20 concentrating the extract and optionally purifying the concentrated extract to obtain a hypoglycemic agent of the present invention.

The present invention may be understood more fully 25 by reference to the following figures, detailed description and illustrative examples which are intended to exemplify non-limiting embodiments of the invention.

4. DESCRIPTION OF THE FIGURES

30 Fig. 1 is a flow-chart describing a method for isolation of a hypoglycemically active fraction from *Cryptolepis* sp.

Fig. 2 is a histogram illustrating the anti-hyperglycemic activity of DF-229-13-4, a fraction from the 35 extract as illustrated in Fig. 1.

Fig. 3 is a histogram illustrating the anti-hyperglycemic activity of DF-229-19-3, a fraction from the extract as illustrated in Fig. 1.

Fig. 4 is a histogram illustrating the anti-
5 hyperglycemic activity of cryptolepine and quindoline in C57BL/ks db mice.

Fig. 5(A-B) is a histogram illustrating the effects of cryptolepine at various doses on plasma glucose levels in: A, diabetic mice and B, lean normal mice.

10 Fig. 6(A-B) is a histogram illustrating the effects of cryptolepine at various doses on triglyceride levels in: A, C57BL/ks db mice, and B, lean normal mice.

Fig. 7 is a histogram illustrating the anti-
hyperglycemic activity of a water extract (roE1) in C57BL/ks
15 db mice.

5. DETAILED DESCRIPTION OF THE INVENTION

5.1 PROCESS FOR PREPARING HYPOGLYCEMICALLY ACTIVE EXTRACTS

20 According to the present invention, a hypoglycemic extract can be prepared from *Cryptolepis* sp., including, but not limited to *C. triangularis* and *C. sanguinolenta*.

According to a first embodiment, a sample of *Cryptolepis* sp. root is washed with a polar alcoholic solvent 25 to extract alkaloid salts therefrom. Suitable alcoholic solvents include, but are not limited to methanol, ethanol, 2-methoxyethanol, 1-propanol, 2-propanol, iso-butanol, sec-butanol and the like. The alcoholic solvents may optionally be diluted with water in order to adjust the polarity and 30 hence the alkaloid salt solubility thereof. The aqueous content of the alcoholic solvents may range from 0-25%, preferably 0-10%. Most preferably, the alcoholic solvent is 90% aqueous ethanol.

The alkaloid salt-containing alcoholic solution is 35 then concentrated so as to remove the alcoholic solvent therefrom. Concentration may optionally occur at elevated

temperatures, in vacuo, or both, so as to expedite the removal of solvent.

The resulting alkaloid salt-containing residue is diluted with water and the resulting alkaloid salt-containing aqueous solution is washed with a non-polar organic solvent to remove neutral extractables therefrom. By "neutral extractables" is meant any non-salts which are soluble in non-polar organic solvents. Suitable non-polar organic solvents which may be used include, but are not limited to diethyl ether, ethyl acetate, isoamyl acetate, benzene, toluene, 2-butanone, 4-methyl-2-pentanone, chlorinated hydrocarbons such as dichloromethane, chloroform, carbon tetrachloride, 1,2-dichloroethane, tetrachloroethylene, and other non-polar organic solvents known to those of ordinary skill in the art.

15 The resulting alkaloid salt-containing aqueous solution is then washed with base to free the alkaloid from its corresponding salt. Such suitable bases include, but are not limited to alkali metal carbonates, such as lithium, sodium, or potassium carbonate, alkali metal bicarbonates, 20 such as lithium, sodium, or potassium bicarbonate, alkali metal hydroxides, such as lithium, sodium, or potassium hydroxide, or mixtures thereof. Preferably, the bases contemplated by the present invention are used as aqueous solutions which may range in concentration from 0.0001 to 12

25 M.

The resulting free base-containing aqueous solution is then extracted with a non-polar organic solvent to give a free base-containing organic solution. The non-polar organic solvent can be chosen from the group of such solvents 30 described above.

The free base-containing organic solution is then concentrated to give a residue comprising alkaloids which are freed from their corresponding salt. Concentration may proceed at room temperature or at an elevated temperature 35 which may accelerate the removal of solvent. In addition, concentration may occur at ambient pressure, or preferably in vacuo.

The free base-containing residue is then purified to provide the hypoglycemically active composition of the present invention. Suitable methods of purification include, but are not limited to recrystallization from solvents and solvent mixtures known to those of skill in the art, ion exchange such as through a column containing DOWEX resin, elution chromatography and combinations thereof. Methods of elution chromatography include, but are not limited to preparative thin-layer chromatography, conventional silica gel chromatography, and high performance liquid chromatography. Purification of the free base-containing residue by any of the above mentioned means may optionally separate the residue into various fractions, each of which may function alone or in combination with any other fraction or fractions as the hypoglycemic agent of the present invention. The hypoglycemic agent of the present invention contains quindoline, cryptolepine or mixtures thereof.

In the event that the hypoglycemic agent obtained from the above purification method contains a mixture of quindoline and cryptolepine, it may be desired to separate the alkaloids into their separate components. In such a case, the hypoglycemic agent may optionally be further purified, by such means as are stated above, to obtain quindoline or cryptolepine in substantially pure form. Preferably, the latter purification step involves high performance liquid chromatography using a water/acetonitrile/isopropanol gradient. According to a preferred embodiment, reverse phase high performance liquid chromatography is performed using C-18 derivatized polymethacrylate (highly cross-linked). According to another embodiment, cross-linked polystyrene is used.

According to a second embodiment of the invention, the hypoglycemic agent is prepared as follows:

Roots of *Cryptolepis* sp. are first washed with a non-polar organic solvent to remove neutral extractables therefrom. By "neutral extractables" is meant any non-salts which are soluble in non-polar organic solvents. Suitable non-polar organic solvents which may be used include, but are

not limited to diethyl ether, ethyl acetate, isoamyl acetate, benzene, toluene, 2-butanone, 4-methyl-2-pentanone, chlorinated hydrocarbons such as dichloromethane, chloroform, carbon tetrachloride, 1,2-dichloroethane, tetrachloroethylene, 5 and other non-polar organic solvents known to those of ordinary skill in the art. Preferably, dichloromethane is used. The root is washed with the non-polar organic solvent primarily to remove any neutral contaminants which may adversely affect the purity and efficacy of the hypoglycemic 10 agent the present invention. Prior to washing, the roots may optionally be ground, for example in a Wiley mill, or otherwise reduced in overall size, so as to increase the effective surface area of the root available to the solvent.

After washing with the non-polar organic solvent, 15 the resulting alkaloid salt-containing marc is wetted with a base, so as to free alkaloids contained therein from corresponding acid salts. Suitable bases which may be used in this capacity include, but are not limited to alkali metal carbonates, such as lithium, sodium, or potassium carbonate, 20 alkali metal bicarbonates, such as lithium, sodium, or potassium bicarbonate, alkali metal hydroxides, such as lithium, sodium, or potassium hydroxide, or mixtures thereof. Preferably, the bases contemplated by the present invention are used as aqueous solutions which may range in concentration 25 from 0.0001 to 12 M. Prior to wetting, the marc may optionally be dried. After wetting with the base, the alkaloids which have been freed from their corresponding acid salts are extracted from the free base-containing marc with a polar alcoholic solvent as described above. The alcoholic 30 solvents may optionally be diluted with water to adjust the polarity thereof. The aqueous content of the alcoholic solvents may range from 0-25%, preferably 0-10%. Preferably, the solvent used to extract from the roots the alkaloids which were freed from their corresponding salts is a mixture of 35 ethanol and water. Most preferably, the solvent system is 90% aqueous ethanol. Prior to extracting, the root may optionally be re-dried, preferably by lyophilizing.

The alcoholic solution containing the alkaloids freed from their corresponding salts is then concentrated to give a residue comprising such alkaloids. Concentration may proceed at room temperature or at an elevated temperature, which may accelerate the removal of solvent. In addition, concentration may occur at ambient pressure, or preferably in vacuo.

The free base-containing residue is then purified to provide the hypoglycemic agent of the present invention. 10 Suitable methods of purification include, but are not limited to recrystallization from solvents and solvent mixtures known to those of skill in the art, ion exchange such as through a column containing DOWEX resin, elution chromatography and combinations thereof. Methods of elution chromatography 15 include, but are not limited to preparative thin-layer chromatography, conventional silica gel chromatography, and high performance liquid chromatography. Purification of the residue by any of the above mentioned means may optionally separate the residue into various fractions, each of which can 20 be screened for hypoglycemic activity. The hypoglycemic agent of the present invention contains quindoline, cryptolepine or mixtures thereof.

In the event that the hypoglycemic agent obtained from the above mentioned purification method contains a 25 mixture of quindoline and cryptolepine, it may be desired to separate the mixture into its separate components. In such a case, the hypoglycemic agent may optionally be further purified, by such means as are stated above, to obtain quindoline or cryptolepine in substantially pure form. 30 Preferably, the latter purification step involves high performance liquid chromatography using a water/acetonitrile/isopropanol gradient. (For an example of this embodiment, see Fig. 1).

According to a third embodiment of the invention, 35 the hypoglycemic agent is prepared by washing *Cryptolepis* sp. roots with a polar alcoholic solvent. For example, 10-25 g of crushed *Cryptolepis* sp. roots are agitated in approximately

1 L of 90% aqueous ethanol for 24-72 hrs. The resulting extract is concentrated to obtain the hypoglycemic agent of the present invention. Suitable polar alcoholic solvents which may be used are those recited above. The alcoholic solvents may optionally be diluted with water to adjust the polarity thereof. The aqueous content of the alcoholic solvents may range from 0-25%, preferably 0-10%. Most preferably, the alcoholic solvent is 90% aqueous ethanol.

The resulting solution is then concentrated, by methods recited above, to obtain a residue. The residue is optionally purified, by methods recited above, to obtain the hypoglycemic agent of the present invention.

In the event that further purification of the hypoglycemic agent obtained from the above method is desired, the hypoglycemic agent may optionally be further purified, by such means as are stated above. Preferably, the latter purification step involves high performance liquid chromatography using a water/acetonitrile/isopropanol gradient.

According to a fourth embodiment of the invention, the hypoglycemic agent is prepared by washing *Cryptolepis* sp. roots with an aqueous acidic solution. For example, 10-100 g of crushed *Cryptolepis* sp. roots are agitated in approximately 1 L of 1-10% acetic acid/water for 24-72 hrs. The resulting extract is neutralized with a base mentioned above and concentrated to obtain the hypoglycemic agent of the present invention. The concentration of such a solution may range from 0.001 to 12 M, preferably from 0.001 to 2 M and most preferably from 0.01 to 1 M. Suitable acids which may comprise the acidic aqueous solution include, but are not limited to carboxylic acids such as formic acid, acetic acid, citric acid, tartaric acid, oxalic acid, malic acid, succinic, ascorbic, gluconic and benzoic, inorganic acids such as hydrochloric acid, sulfuric acid, hydrobromic acid, hydroiodic acid, and orthophosphoric acid, phosphoric and other acids capable of forming stable alkaloid salts commonly known to

those skilled in the art. Preferably, aqueous acetic acid is used.

The resulting alkaloid salt-containing aqueous solution is then concentrated, by methods recited above, to obtain an alkaloid salt-containing residue. Prior to concentration, the alkaloid salt-containing aqueous solution is washed with base, as recited above, to free the alkaloid from its corresponding salt and extracted with a non-polar organic solvent, as recited above, to give a free base-containing organic solution. After concentration, the residue is optionally purified, by methods recited above, to obtain the hypoglycemic agent of the present invention.

The hypoglycemic agent may optionally be further purified, by such means as are recited above. Preferably, the latter purification step involves high performance liquid chromatography using a water/acetonitrile/isopropanol gradient.

According to a fifth embodiment of the invention, the hypoglycemic agent is prepared by extracting *Cryptolepis* sp. roots with water. For example, 1.0-2.0g of crushed *Cryptolepis* sp. roots are steeped in approximately 250 mL of water heated at 55-65°C for 5 minutes-24 hrs. The resulting extract comprises the hypoglycemic agent of the present invention. Suitable extraction temperatures range from 25-100°C, preferably 45-65°C, and most preferably 55-65°C.

The resulting aqueous extract may optionally be concentrated, to obtain a concentrate having hypoglycemic activity. The concentrate can be further dehydrated for example, lyophilized or spray dried to form a composition which upon rehydration with water, aqueous or other pharmaceutically acceptable excipient, can be used as a hypoglycemic agent. The concentrate is optionally purified, by methods described above, to obtain a hypoglycemic agent for use in the methods of the present invention. Prior to concentration, the extract can optionally be washed with base and/or extracted with a non-polar organic solvent as recited above.

It may be desired to separate the mixture into its separate components. In such a case, the hypoglycemic agent may optionally be further purified, by such means as are stated above, to obtain quindoline or cryptolepine in substantially pure form. Preferably, the latter purification step involves high performance liquid chromatography using a water/acetonitrile/isopropanol gradient.

5.2 ISOLATION OF QUINDOLINE, CRYPTOLEPINE OR MIXTURES THEREOF

10 The extracts of the present invention may be further purified so as to isolate a more pure extract or quindoline, cryptolepine or mixtures thereof from the extract. Preferably, the extract is purified by ion exchange with a highly acidic resin such as DOWEX followed by high-performance 15 liquid chromatography over a neutral polystyrene matrix, thereby isolating from the extract quindoline, cryptolepine or mixtures thereof. Most preferably, the high-performance liquid chromatography column is eluted with water/acetonitrile/isopropanol. According to a preferred 20 embodiment, reverse phase high performance liquid chromatography is performed using C-18 derivatized polymethacrylate (highly cross-linked). According to another embodiment, cross-linked polystyrene is used. As contemplated by the present invention, quindoline, cryptolepine or mixtures 25 thereof may be used directly as hypoglycemic agents.

In a preferred mode of extraction, the roots of *Cryptolepis sanguinolenta* are extracted with a solution of 1% acetic acid in water so described by Ablordeppay et al.

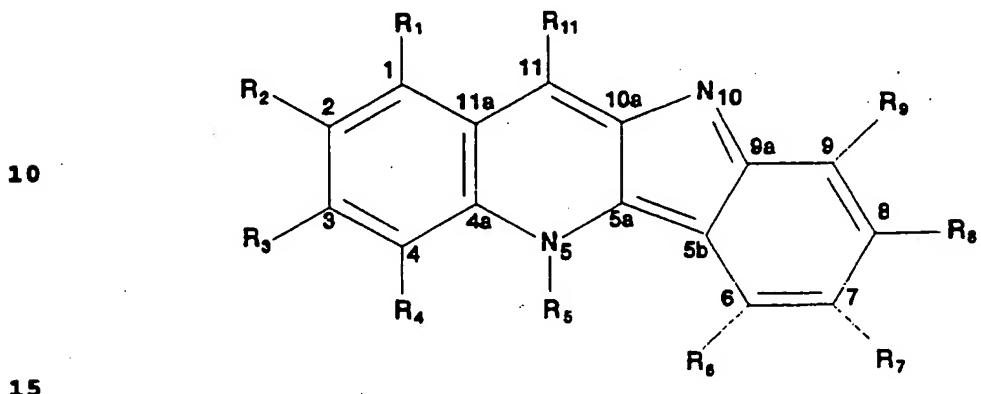
30 (Planta Medica, 56, 416 (1990)).

35 Alternatively, quindoline or cryptolepine can be obtained by methods of classical organic synthesis known by those of skill in the art (see Fichte, F. and Boehringer, R., Chem. Ber., 39, 3932 (1906)) for use according to the methods of the invention.

5.3 OTHER QUINDOLINE ALKALOIDS

According to the present invention, quindoline alkaloids having the generic structure below are also useful as hypoglycemic agents:

5



(a) where $R_1 - R_4$ and $R_6 - R_{11} = H$, and R_5 is selected from the group of ethyl, isopropyl, benzyl, 4-hydroxybenzyl, 4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl, 3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl, 3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, 4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino, 2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;

(b) where R_1, R_4 and $R_6 - R_{11} = H$, $R_2 = R_3 = -CH_2-O-CH_2-$, and R_5 is selected from the group consisting of ethyl, isopropyl, benzyl, 4-hydroxybenzyl,

4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl, 3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl, 3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, 4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino, 2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl, 10 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;

(c) where $R_1 - R_4$, $R_6 - R_7$ and $R_{10} - R_{11} = H$, $R_8 = R_9 = -CH_2-O-CH_2-$, and R_5 is selected from the group consisting of ethyl, isopropyl, benzyl, 4-hydroxybenzyl, 4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl, 20 3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl, 3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, 25 4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino, 2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;

(d) where $R_1 - R_3$ and $R_6 - R_{11} = H$, and $R_4 - R_5 = -CH_2CH_2-$;

(e) where $R_1 - R_3$ and $R_6 - R_{11} = H$, and $R_4 - R_5 = -CH_2CH_2CH_2-$;

(f) where $R_1 - R_4$ and $R_6 - R_{11} = H$, $R_5 = CH_3$, and 10a, 11 = dihydro;

5 (g) where $R_1 - R_4$ and $R_6 - R_{11} = H$, $R_5 = CH_3$, and 5a, 5b = dihydro;

(h) where $R_1 - R_4$ and $R_6 - R_{11} = H$, $R_5 = CH_3$, and 5a, 5b, 10a, 11 = tetrahydro.

10 (i) where $R_1 - R_{11} = H$, and 9a, 10 = dihydro; and (j) where $R_1 - R_{11} = H$, and 10-methyl.

Compounds of the above structure can be prepared by several routes. For example, a suitably substituted anthranilic acid derivative may be condensed with an analogously substituted 3-hydroxyindole in the presence of 15 $Ba(OH)_2$, followed by decarboxylation in the presence of potassium hydroxide and zinc to give substituted quindoline alkaloids (see, E. Giraud, Compt. Rend., 85, 104 (1879) and F. Fichter and B. Rohner, Chem. Ber., 43, 3489 (1910)).

20 Alternatively, a substituted 1,2-(o-nitrophenyl)-1-cyanoethane may be heated in the presence of $(NH_4)_2S$ in alcohol to further obtain substituted quindoline alkaloids (see, P. Gabriel and H. Eschenbach, Chem. Ber., 30, 3020 (1897)).

Substituted quindoline alkaloids may also be obtained from substituted bis(o-nitrobenzyl) malonates which, 25 after decarboxylation with alkali, may be heated in the presence of HI/P (F. Fichter and R. Boehringer, Chem. Ber., 39, 3932 (1906)).

Dihydroquindolines may be obtained by reducing appropriately substituted quindoline alkaloids with tin in HCl 30 (see, F. Fichter and R. Boeringer, Chem. Ber., 39, 3932 (1906)).

Additionally, other routes to substituted quindolines may be applied (see, J.W. Armit and R.J. Robinson, J. Chem. Soc., 121, 827 (1922); S.J. Holt and V. Petrow, J. Chem. Soc., 607 (1947); E. Gellert, Raymond-Hamet and E. Schlittler, Helv. Chim. Acta, 34, 642 (1951); K.E. Schulte, J.

Reisch and U. Stoess, Arch. Pharmaz., 305, 523 (1972); V.P. Sevodin, V.S. Velezhera, Y.V. Erofeav, N.N. Suvorov Khim. Geterotsikl. Soedin., 1667 (1984); A. Buzas, J.Y. Merour, Synthesis, 458 (1989); Y.A. Degutis, A.B. Ezyarskaite, Khim. Geterotsikl. Soedin., 1375 (1986); Y. Takeuchi, M.R. Chang, K. Hasigaki, T. Tashiro, T. Tsuro, S. Tsukagoshi, M. Yamato, Chem. Pharm. Bull., 40, 1481 (1992); M.R. Chang, Y. Takeuchi, K. Hashigaki, M. Yamato, Heterocycles 33, 147 (1992); and M. Yamato, Y. Takeuchi, M.R. Chang, K. Hashiyaki, Chem. Pharm. Bull., 40, 528 (1992).

The invention also encompasses novel compounds of formula I and pharmaceutically acceptable salts thereof, as well as compositions comprising such compounds and methods for their use.

15

5.4 METHODS FOR USE OF EXTRACTS OF CRYPTOLEPIS SP. AND QUINDOLINE ALKALOIDS SUCH AS CRYPTOLEPINE AND QUINDOLINE

Due to the potent activity of the extracts of *Cryptolepis* sp., obtained, as described in section 5.2 above, using an organic solution, a polar alcoholic solution, an aqueous acidic solution or an aqueous solution, as hypoglycemic agents (see Section 7, infra), the extracts are advantageously useful in veterinary and human medicine for therapeutic treatment of diabetes mellitus. Additionally, the extracts can be advantageously be used as hypoglycemic agents to reduce the blood glucose level in situations of acute stress such as experienced by animals or patients with hyperthermia, trauma, sepsis, and burns and undergoing general anesthesia. Hyperglycemia sometimes associated with severe head injury, cerebral thrombosis, encephalitis and heat stroke can also be therapeutically treated with these biologically active extracts. Additionally, the extracts are useful as hypoglycemic agents for rare congenital metabolic glycogen storage disease associated with hyperglycemia.

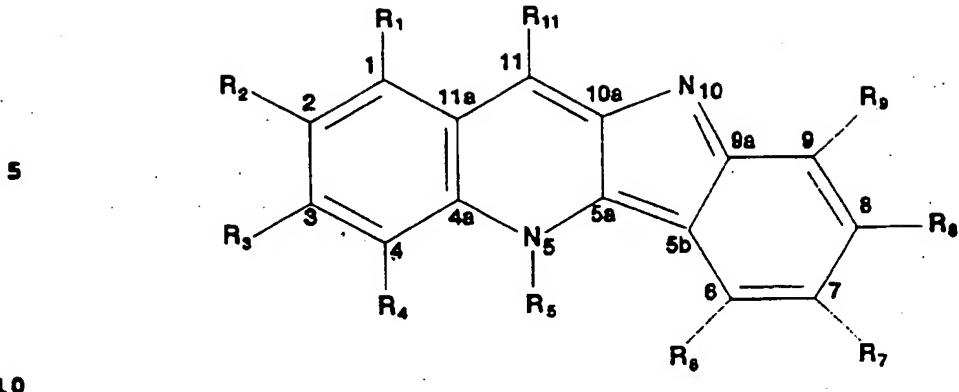
35 Although the present inventors do not wish to be limited to any particular mechanism of action to explain the

hypoglycemic activity of the hypoglycemic agents of the present invention, it is envisaged that the extracts or the quindoline or cryptolepine may advantageously be useful for treatment of both insulin-dependent or type I diabetes (5 formerly termed juvenile-onset or ketosis-prone diabetes) and non-insulin-dependent or type II diabetes (formerly termed adult-onset, maturity-onset or nonketotic diabetes).

When administered to a mammal for veterinary use or to a human for clinical use, the extracts can be used alone, (10) or may be combined with any physiologically acceptable carrier such as water, an aqueous solution, normal saline, or other physiologically acceptable excipient. In general, the dosage would range from about 10-1000 mg/kg/day, preferably about 10-250 mg/kg/day.

(15) The extracts can be administered by a number of routes, including, but not limited to: orally, injection including, but not limited to intravenously, intraperitoneally, subcutaneously, intramuscularly, etc. The preferred route of administration is oral. Additionally, the (20) extracts can be administered in conjunction with another hypoglycemic including such as insulin; a biguanide such as metformin or buformin; a sulfonylurea such as acetohexamide, chlorpropamide, tolazamide, tolbutamide, glyburide, glypizide or glyclazide; a thiazolidinedione such as troglitazone; an α -glycosidase inhibitor such as acarbose or miglitol; or a β -adrenoceptor agonist such as CL-316, 243, etc.

According to another embodiment of the invention, compositions comprising a quindoline alkaloid compound having the following structure or a pharmaceutically acceptable salt (25) thereof



I

said compound selected from group consisting of:

- (a) where $R_1 - R_{11} = H$;
- 15 (b) where $R_1 - R_4$ and $R_6 - R_{11} = H$, and $R_5 = CH_3$;
- (c) where $R_1 - R_4$ and $R_6 - R_{11} = H$, and R_5 is selected from the group of ethyl, isopropyl, benzyl, 4-hydroxybenzyl, 4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl, 3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl, 20 3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, 4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino, 25 2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;
- (d) where R_1, R_4 and $R_6 - R_{11} = H$, $R_2 = R_3 = -CH_2-O-CH_2-$, and R_5 is selected from the group consisting of ethyl, isopropyl, benzyl, 4-hydroxybenzyl, 4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl, 3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl, 3-fluorophenyl, 2-

iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, 4-chlorophenyl,
5 2-pyridino, 3-pyridino, 4-pyridino, 2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-fluorobenzyl, 3-fluorobenzyl,
10 4-fluorobenzyl, 2-iodobenzyl, 3-iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;

(e) where $R_1 - R_4$ and $R_6 - R_7$ and $R_{10} - R_{11} = H$, $R_8 = R_9$,
15 $R_5 = -CH_2-O-CH_2-$, and R_5 is selected from the group consisting of ethyl, isopropyl, benzyl, 4-hydroxybenzyl, 4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl, 3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl, 3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, 4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino, 2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-fluorobenzyl, 3-fluorobenzyl, 2-iodobenzyl, 3-iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;

(f) where $R_1 - R_3$ and $R_6 - R_{11} = H$, and $R_4 - R_5 = -CH_2CH_2-$;
30 (g) where $R_1 - R_3$ and $R_6 - R_{11} = H$, and $R_4 - R_5 = -CH_2CH_2CH_2-$;

(h) where $R_1 - R_4$ and $R_6 - R_{11} = H$, $R_5 = CH_3$, and 10a, 11 = dihydro;
 (i) where $R_1 - R_4$ and $R_6 - R_{11} = H$, $R_5 = CH_3$, and 5a, 5b = dihydro;
 (j) where $R_1 - R_4$ and $R_6 - R_{11} = H$, $R_5 = CH_3$, and 5a, 5b, 10a, 11 = tetrahydro.
 (k) where $R_1 - R_{11} = H$, and 9a, 10 = dihydro; and
 (l) where $R_1 - R_{11} = H$, and 10-methyl
 are advantageously useful in veterinary and human medicine as
 10 hypoglycemic agents.

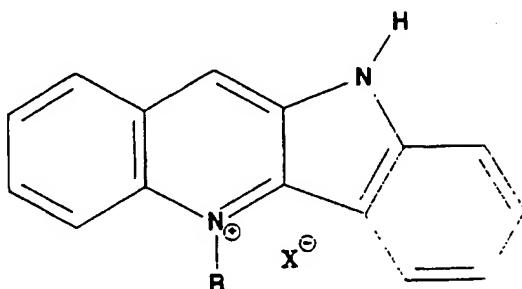
A preferred mode of this embodiment of the invention
 encompasses methods of administering the cryptolepine or
 quindoline as hypoglycemic agents.

The quindoline alkaloids, including quindoline or
 15 cryptolepine, can be administered in an effective amount
 either as alkaloids freed from their corresponding salts or
 pharmaceutically acceptable salts such as hydrochloride,
 hydrobromide, phosphate, sulfate, acetate, succinate,
 ascorbate, tartrate, gluconate, benzoate, malate, and
 20 fumarate.

The pharmaceutically acceptable salts of
 cryptolepine and quindoline which are useful for the present
 invention having the following structure:

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$R = H$, quindoline
 $R = CH_3$, cryptolepine
 X^- = conjugate base of acid.

When administered to a mammal for veterinary use or to a human for clinical use, a quindoline alkaloid compound of formula I, including but not limited to quindoline and cryptolepine, or pharmaceutically acceptable salts thereof may 5 be used alone, or may be combined with any physiologically acceptable carrier such as water, an aqueous solution, normal saline, or other physiologically acceptable excipient. In general, the dosage would range from about 1 to about 100 mg/kg body weight of the human or animal to be treated.

10 The quindoline alkaloid compounds of formula I, including quindoline and cryptolepine, or pharmaceutically acceptable salts thereof can be administered by a number of routes, including, but not limited to: orally, injection including, but not limited to intravenously, 15 intraperitoneally, subcutaneously, intramuscularly, etc. The preferred route of administration is oral. Additionally, the quindoline alkaloid compounds of formula I, including quindoline and cryptolepine, or a pharmaceutically acceptable salt thereof, can be administered in conjunction with another 20 hypoglycemic including such as insulin; a biguanide such as metformin or buformin; a sulfonylurea such as acetohexamide, chlorpropamide, tolazamide, tolbutamide, glyburide, gliclazide or gliclazide; a thiazolidinedione such as troglitazone; an α -glycosidase inhibitor such as acarbose or miglitol; or β - 25 adrenoceptor agonist such as CL-316, 243, etc.

Finally, the quindoline alkaloid compounds of formula I, including quindoline and cryptolepine, or pharmaceutically acceptable salts thereof and compositions containing said quindoline alkaloid compounds of formula I, 30 including quindoline and cryptolepine, or pharmaceutically acceptable salts thereof can be used for research purposes, for example, to investigate the mechanism and activity of hypoglycemic agents.

The following series of Examples are presented by 35 way of illustration and not by way of limitation on the scope of the invention.

6. PREPARATION OF BIOLOGICALLY ACTIVE EXTRACTS

Dried root of *Cryptolepis sanguinolenta* was obtained and an extract prepared according to one embodiment of the present invention as illustrated in Fig. 1. 1 Kg of the root material was finely ground in a Wiley mill and macerated overnight with 10 L of dichloromethane (DCM). After filtration, the dichloromethane extract was evaporated to dryness to yield 22.7g of neutral extractables (2.26 wt. %) (herein designated as fraction DF-229-13-1). The remaining root powder was dried and subsequently wetted with 10% aqueous sodium carbonate solution until the material gained dough consistence. The wetted root material was then lyophilized for three days until powder dry. This material was macerated under gentle stirring for 24 hrs. with 90% aqueous ethanol, filtered, and the filtrate reduced to dryness under vacuum (herein designated as fraction DF-229-13-4). (This extract contained the alkaloids as free bases and showed pronounced antihyperglycemic effect at 250 mg/kg in the C57BL/ks db mouse model). 5 g of fraction DF-229-13-4 were loaded on a chromatography column filled with DOWEX 50X8-400 strongly acidic ion exchange resin and washed subsequently with 10% aqueous ethanol, 95% ethanol and 10% ammonia in methanol to give after evaporation of solvents 1.95 g (39 wt. %) of aqueous ethanol eluate (herein designated as fraction DF-229-19-1), 0.3 g (6 wt. %) of ethanol eluate (herein designated as fraction DF-229-19-2), and 2.3g (46 wt. %) of methanolic ammonia eluate (herein designated as fraction DF-229-19-3). Proton and carbon nuclear magnetic resonance spectroscopy (NMR) of the fraction DF-229-19-3 showed that the fraction contained cryptolepine and quindoline in a ratio of approximately 1:1. Fraction DF-229-19-3 was then loaded onto a highly cross-linked polymethacrylate HPLC column and separated following gradient elution by a water/acetonitrile/isopropanol gradient. More particularly, the HPLC column used over a YMC-Pack 250 mm x 4.6 mm Polymer

C18 6 μ m pore size. The flow rate was 1 ml/min. The solvent system is shown below in Table 1.

TABLE 1

5 **HPLC SOLVENT SYSTEM**

Time (min)	% H ₂ O	% Acetonitrile	% Isopropanol
00.0	80	20	0
10 13.00	100	0	0*
15.00	100	0	0
15.10	0	30	70*
25.20	0	30	70

15

* Linear Gradient

Detection used was: Rainin UV-1 detector at 254 nm.

Retention time of Peak I was 12.3 min.; Peak II, 14.4 min.

20 (See Fig. 1). Although not desiring to be limited to a particular structure for the hypoglycemic agent of Peaks I and II, the NMR spectra of these peaks are consistent, respectively, with cryptolepine and quindoline. Two peaks, corresponding, respectively, to cryptolepine and quindoline in 25 a 1:1 ratio, respectively, were collected, concentrated and evaluated for biological activity.

While ¹H NMR analysis of fraction DF-229-19-3 (infra) suggested a mixture comprised primarily of cryptolepine, the present inventors do not wish to be limited 30 to the use of compounds having the chemical structure of quindoline or cryptolepine. As demonstrated in Section 7 (infra), DF-229-19-3 has hypoglycemic activity associated with the compounds corresponding to peaks I and II.

35 According to another embodiment of the invention, an extract was prepared as follows. 100 g of dried root of *Cryptolepis sanguinolenta*, in ground or powdered form was

percolated overnight, at about 55°C, in 1 L of water. After filtration, the aqueous extract was evaporated to dryness to yield a hypoglycemic extract of the present invention.

5 7. HYPOGLYCEMIC AND OTHER EFFECTS OF EXTRACTS

The following experiments demonstrate that the extracts of the present invention and quindoline alkaloids, for example, cryptolepine and quindoline, and salts thereof produce a significant and consistent hypoglycemic effect on 10 obese diabetic mice, i.e., an art recognized model of diabetes mellitus. The experiments also demonstrate reduction of plasma triglyceride in diabetic mice.

7.1 MATERIALS AND METHODS

15

Animals

Genetically transformed diabetic male mice (C57BL/ks db) (Jackson Laboratories, Bar Harbor, Maine) of 8-9 weeks of age served as test animals. In some experiments, littermate lean mice (non-diabetic mice) were also included as test 20 animals. Five animals per cage were housed at 22±3°C at 50±20% relative humidity. The mice were fed a diet of Purina rodent chow, with water ad libitum. The mice were identified via cage labeling and tail marking.

25

Methods

Prior to dosing, 20µL of blood were collected from each animal by tail nick. Plasma glucose levels were determined by the glucose oxidase method colorimetrically (Sigma test kit, cat.# 315, Sigma Chemical Co., St. Louis, MO). These values were used as predose levels. Animals with plasma glucose levels below 300 mg/dL were eliminated from the study, while the remaining ones were randomized into groups of 30 with mean glucose levels of not more than 20 mg/dL of difference. Body weight and food consumption were measured 35 daily during the experiment period.

Extracts or purified compounds were either dissolved or suspended in liquid solvent as indicated and administered, orally to test animals using a 20 gauge feeding needle and a 1 mL syringe. The dose volume was 10 mL/kg. Blood samples were collected at specified intervals. Plasma glucose levels were measured immediately after bleeding. In some experiments, plasma triglyceride levels were measured using an enzymatic colorimetric assay (Triglyceride Diagnostic Kit, Sigma, cat. #339 (Sigma Chemical Co., St. Louis, MO).

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7.2 EXPERIMENTS

Animal experiments were conducted using fractions of an extraction of *Cryptolepis* sp. as illustrated in Fig. 1, generally as described in Section 7.1. In one experiment, DF-229-13-4 and metformin were separately suspended in aliquots of 0.25 wt % carboxymethyl cellulose + 1% TWEEN™ 60 (Vehicle). Three groups of 5 animals (C57BL/ks db mice) were used. DF-229-13-4 was administered at 250 mg/kg at 0 hr and at 100 mg/kg at 24 hrs.; metformin at 250 mg/kg at 0 hr and 24 hrs (positive control); Vehicle at 10 mL/kg at 0 hr and 24 hrs (negative control). The results are shown in Fig. 2.

As illustrated, administration of fraction DF-229-13-4, administered at 250 mg/kg at 0 hrs. and at 100 mg/kg at 24 hrs., reduced the plasma glucose from a basal level of approx. 440 mg/dL to approx. 300 mg/dL after 3 hrs. and approx. 250 mg/dL after 24 hrs.

In another experiment, fractions DF-229-19-1, DF-229-19-2 and DF-229-19-3 (see Fig. 1) were administered as described above at 100 mg/kg. Vehicle (0.25% carboxymethyl cellulose + 1% TWEEN™ 60) served as the negative control. Plasma glucose was tested at 0, 3, 24 and 27 hrs. The results are presented in Fig. 3.

Fraction DF-229-19-3, the most active, reduced the plasma glucose from a basal level of approx. 435 mg/dL to approx. 262 mg/dL after 24 hrs. and to approx. 197 mg/dL after 27 hrs. Fraction DF-229-19-2 was also active in lowering

glucose levels but its pattern of activity was different from that of fraction DF-229-19-3.

In another experiment hypoglycemic effects of cryptolepine and quindoline, obtained as Peaks I and II 5 illustrated in Fig. 1 (see Section 6 above), were assessed generally as described in Section 7.1. Four groups of experimental animals were included. Group A, negative control received 0.25% carboxymethyl cellulose (CMC); Group B, positive control, 250 mg/kg metformin; Group C, cryptolepine, 10 100 mg/kg; and Group D, quindoline, 100 mg/kg, via oral administration. Blood glucose was tested at 0, 3 and 24 hrs. post-administration. The results are shown in Fig. 4.

As demonstrated in Fig. 4, both cryptolepine and quindoline, administered at 100 mg/kg, are more effective than 15 metformin, administered at 250 mg/kg, in reducing plasma glucose levels after 24 hrs. Cryptolepine and quindoline reduced the plasma glucose from a basal level of approx. 475 mg/dL to approx. 325 mg/dL, whereas with metformin, the plasma glucose level returned to the basal value after 24 hrs.

20 In another experiment, the hypoglycemic effect of various doses of cryptolepine was evaluated in genetically transformed obese diabetic male mice (C57BL/ks db) and their littermate lean, non-diabetic mice 8 weeks of age, obtained from Jackson Laboratories, Bar Harbor, Maine.

25 Vehicle (0.25% CMC +1% TWEEN™ 60) was administered orally as a negative control; metformin, at 250 mg/kg as a positive control, and cryptolepine, at various doses, i.e., 10, 30 and 100 mg/kg. All compositions were administered in 0.25% CMC + 1% TWEEN™ in deionized water. Each solution was 30 sonicated for 1 minute with a Bransonic ultrasonic tip. The dosage was via oral route. The dosing volume was 10 mL/kg body weight.

All compositions were administered at 0, 24 and 48 hrs. Blood samples were collected at 0, 3, 24, 27, 48 and 51 35 hours. Plasma glucose and triglyceride levels were measured immediately after bleeding.

Results are presented in Figs. 5 (A-B) for plasma glucose levels and 6 (A-B) for triglyceride levels in both diabetic and normal animals.

The results in Fig. 5A show that the administration of cryptolepine to diabetic (C57BL/ks db) mice at 30 mg/kg resulted in approximately the same reduction of plasma glucose levels as a 250 mg/kg administration of metformin. Moreover, a 100 mg/kg administration of cryptolepine lowered the blood sugar from a basal value of approx. 425 mg/dL to approx. 250 mg/dL after 24 hrs. and to approx. 225 mg/kg after 27 hrs.

Results in Fig. 5B show that administration of 30 mg/kg of cryptolepine to normal lean mice did not affect the blood sugar. Although 100 mg/kg of cryptolepine lowered the blood glucose from a basal value of approx. 140 mg/dL to approx. 110 mg/dL after 24 hrs., further dosing did not affect the glucose level. Thus, the results indicate that cryptolepine significantly lowered glucose levels and gives a dose-dependent anti-diabetic effect.

The results shown in Fig. 6A indicate that after 3 hrs., cryptolepine administered to diabetic C57BL/ks db mice at a level of 100 mg/kg lowered triglyceride levels to 30% of basal triglyceride levels, as compared to only 50% with a 250 mg/Kg administration of metformin. In addition, an Analysis of Variance was used to analyze the data presented in Fig. 6A. A comparison between treatment groups and the negative control was made. The results indicate that cryptolepine and metformin both significantly lowered the triglyceride levels.

After 3 hrs., cryptolepine administered at 100 mg/Kg to lean non-diabetic mice (see Fig. 6B) resulted in a change of plasma triglyceride levels comparable to those with metformin at 250 mg/Kg. An Analysis of Variance indicated, however, that none of the treatments significantly affected triglyceride levels in the non-diabetic animals.

In yet another series of experiments, hypoglycemic effects of aqueous extracts of the present invention were demonstrated using genetically transformed obese diabetic male mice (C57BL/ks db) of 8 weeks of age, obtained from Jackson

Laboratories, Bar Harbor, Maine. The experiments were conducted generally as described above in Section 7.1. The aqueous extract was prepared as described in Section 6, above.

Group A, negative control received 0.25 wt.% CMC; 5 Group B, positive control, metformin at 250 mg/kg; Group C, aqueous extract, at 250 mg/kg; and Group D, aqueous extract, at 1000 mg/kg. All compositions were administered via oral route in 0.25 wt.% CMC in deionized water.

The dosing time was daily from day 1 to day 15. 10 Blood samples were collected at day 0, 3, 8, 12 and 15, 3 hrs. after daily dosing. An additional blood sample was collected on day 8 at 6 hrs. after dosing. Plasma glucose levels were measured immediately after bleeding. For this study, the number of animals used (N) = 8.

15 Results are presented in Fig. 7.

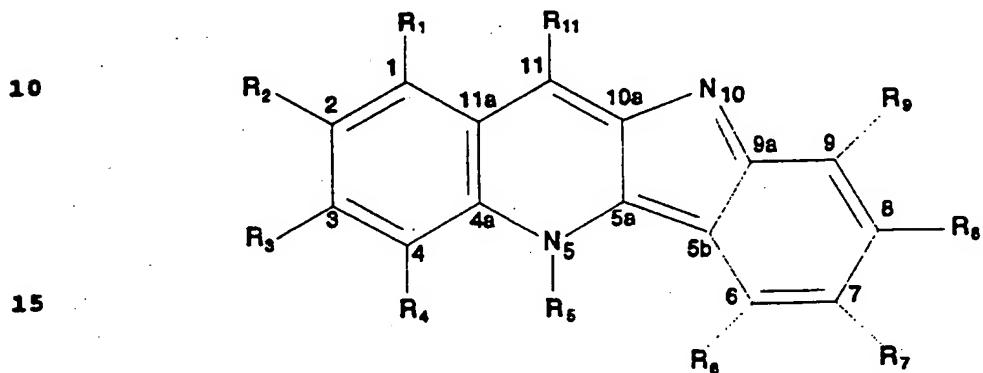
The results illustrated in Fig. 7 show that the administration of the aqueous extract to C57BL/ks db mice at 250 mg/Kg resulted in approximately the same reduction of plasma glucose levels as a 250 mg/Kg administration of 20 metformin. Moreover, a 250 mg/Kg administration of the aqueous extract lowered the blood sugar from a basal value of approx. 550 mg/dL to approx. 425 mg/dL after 12 days. These results are comparable with those received obtained using metformin administered at 250 mg/Kg.

25 The present invention is not to be limited in scope by the specific embodiments disclosed in the examples which are intended as illustrations of a few aspects of the invention and any embodiments which are functionally equivalent are within the scope of this invention. Indeed, 30 various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art and are intended to fall within the appended claims.

A number of references have been cited and the 35 entire disclosures of which are incorporated herein by reference.

What is claimed is:

1. A method for reducing the blood glucose of a mammal, comprising administering to said mammal a hypoglycemically effective amount of a composition comprising a compound having the following structure or a pharmaceutically acceptable salt thereof



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20 said compound selected from group consisting of:

25 (a) where $R_1 = R_{11} = H$;
(b) where $R_1 = R_4$ and $R_6 = R_{11} = H$, and $R_5 = CH_3$;
(c) where $R_1 = R_4$ and $R_6 = R_{11} = H$, and R_5 is selected from the group of ethyl, isopropyl, benzyl, 4-hydroxybenzyl, 4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl, 3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl, 3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, 4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino, 2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-

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iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;

(d) where R_1 , R_4 R_6 - R_{11} = H, R_2 = R_3 = $-CH_2-O-CH_2-$,

5 and R₁ is selected from the group consisting of ethyl, isopropyl, benzyl, 4-hydroxybenzyl, 4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl, 3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl, 3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, 4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino, 2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;

(e) where $R_1 = R_4$, $R_6 = R_7$ and $R_{10} = R_{11} = H$, $R_8 = R_9 = -CH_2-O-CH_2-$, and R_5 is selected from the group consisting of ethyl, isopropyl, benzyl, 4-hydroxybenzyl,
 25 4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl, 3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl, 3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, 4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino, 2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-

pyridylm thyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;

(f) where $R_1 - R_3$, $R_6 - R_{11} = H$, and $R_4 - R_5 = -CH_2CH_2-$;

5 (g) where $R_1 - R_3$, $R_6 - R_{11} = H$, and $R_4 - R_5 = -CH_2CH_2CH_2-$;

(h) where $R_1 - R_4$ and $R_6 - R_{11} = H$, $R_5 = CH_3$, and 10a, 11 = dihydro;

10 (i) where $R_1 - R_4$ and $R_6 - R_{11} = H$, $R_5 = CH_3$, and 5a, 5b = dihydro;

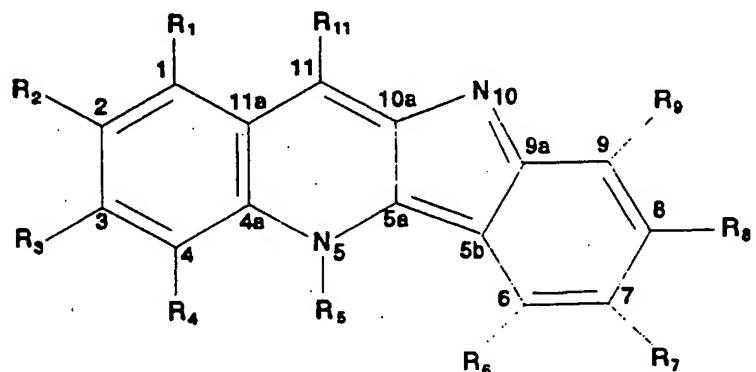
(j) where $R_1 - R_4$ and $R_6 - R_{11} = H$, $R_5 = CH_3$, and 5a, 5b, 10a, 11 = tetrahydro;

(k) where $R_1 - R_{11} = H$, and 9a, 10 = dihydro; and

(l) where $R_1 - R_{11} = H$, and 10-methyl.

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2. A method for treatment of diabetes mellitus, comprising administering, to a mammal suffering from diabetes mellitus, a therapeutically effective amount of a composition comprising a compound having the following structure or a 20 pharmaceutically acceptable salt thereof



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(a) where $R_1 - R_{11} = H$;

(b) where $R_1 - R_4$ and $R_6 - R_{11} = H$, and $R_5 = CH_3$;

35 (c) where $R_1 - R_4$ and $R_6 - R_{11} = H$, and R_5 is selected from the group of ethyl, isopropyl, benzyl, 4-hydroxybenzyl,

4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl, 3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl, 3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, 4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino, 2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl, 10 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;

(d) where $R_1, R_4, R_6 - R_{11} = H$, $R_2 = R_3 = -CH_2-O-CH_2-$, and R_5 is selected from the group consisting of ethyl, isopropyl, benzyl, 4-hydroxybenzyl, 4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl, 3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl, 3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, 4-chlorophenyl, 4-hydroxyphenyl, 2-pyridino, 3-pyridino, 4-pyridino, 2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;

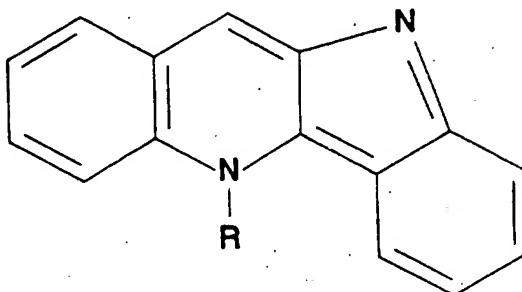
(e) where $R_1 - R_4, R_6 - R_7$ and $R_{10} - R_{11} = H$, $R_8 = R_9 = -CH_2-O-CH_2-$, and R_5 is selected from the group consisting of ethyl, isopropyl, benzyl, 4-hydroxybenzyl, 4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl,

3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl,
3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-
hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-
dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-
5 methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl,
4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino,
2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-
dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl,
3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-
10 chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-
fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-
iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-
pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-
bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;
15 (f) where $R_1 = R_3$, $R_6 = R_{11} = H$, and $R_4 = R_5 =$
-CH₂CH₂-;
(g) where $R_1 = R_3$, $R_6 = R_{11} = H$, and $R_4 = R_5 =$
-CH₂CH₂CH₂-;
(h) where $R_1 = R_4$ and $R_6 = R_{11} = H$, $R_5 = CH_3$, and
20 10a, 11 = dihydro;
(i) where $R_1 = R_4$ and $R_6 = R_{11} = H$, $R_5 = CH_3$, and 5a,
5b = dihydro;
(j) where $R_1 = R_4$ and $R_6 = R_{11} = H$, $R_5 = CH_3$, and 5a,
5b, 10a, 11 = tetrahydro;
25 (k) where $R_1 = R_{11} = H$, and 9a, 10 = dihydro; and
(l) where $R_1 = R_{11} = H$, and 10-methyl.

3. A method for reducing the blood glucose of a
mammal, comprising administering to said mammal a
30 hypoglycemically effective amount of a composition comprising
a compound having the following structure or a

pharmacutically acceptable salt thereof

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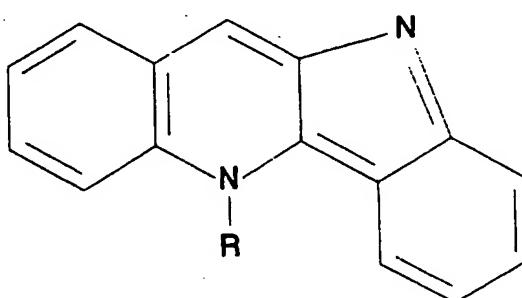
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where R = H or CH₃.

4. The method of claim 3, in which the pharmaceutically acceptable salt is selected from the group consisting of hydrochloride, hydrobromide, phosphate, sulfate, acetate, succinate, ascorbate, tartrate, gluconate, benzoate, malate and fumarate.

5. A method for treatment of diabetes mellitus,
20 comprising administering, to a mammal suffering from diabetes mellitus, a therapeutically effective amount of a composition comprising a compound having the following structure or a pharmaceutically acceptable salt thereof

25



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where R = H or CH₃.

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6. The method of claim 5, in which the pharmaceutically acceptable salt is selected from the group

consisting of hydrochloride, hydrobromide, phosphate, sulfate, acetate, succinate, ascorbate, tartrate, gluconate, benzoate, malate and fumarate.

5 7. The method according to claim 5, in which the composition is administered in conjunction with another hypoglycemic agent selected from the group consisting of a sulfonylurea, a biguanide, a thiazolidinedione, a β -adrenoceptor agonist, an α -glucosidase inhibitor and insulin.

10 8. The method according to claim 7, in which the sulfonylurea is selected from the group consisting of acetohexamide, chlorpropamide, tolazamide, tolbutamide, glyburide, gliclazide and gliclazide.

15 9. The method according to claim 7, in which the biguanide is metformin or buformin.

10. The method according to claim 7, in which the 20 α -glucosidase inhibitor is acarbose or miglitol.

11. The method according to claim 7, in which the thiazolidinedione is troglitazone.

25 12. A method for reducing the blood glucose of a mammal, comprising administering to a mammal in need of such blood glucose reduction, an effective amount of an extract from a *Cryptolepis* sp. in which said extract was obtained by a process which comprised:

30 (a) washing a root of a *Cryptolepis* sp. with an alcoholic solvent to obtain an alkaloid salt-containing alcoholic solution; and

35 (b) concentrating the alkaloid salt-containing alcoholic solution to obtain an alkaloid salt-containing residue useful as a hypoglycemic agent.

13. The method according to claim 12, in which the extract was obtained by a process which further comprised:

(c) diluting the alkaloid salt-containing residue with water to obtain an alkaloid salt-containing aqueous solution;

(d) washing the alkaloid salt-containing aqueous solution with a non-polar organic solvent to remove neutral extractables therefrom;

10 (e) washing the alkaloid salt-containing aqueous solution of step (d) with base to obtain a free base-containing aqueous solution;

(f) extracting the free base-containing aqueous solution with a non-polar organic solvent to obtain a free base-containing organic solution; and

15 (g) concentrating the free base-containing organic solution to obtain a free base-containing extract.

14. A method for treatment of diabetes mellitus, comprising administering, to a mammal suffering from diabetes mellitus, a therapeutically active amount of an extract from a *Cryptolepis* sp., in which said extract was obtained by a process which comprised:

(a) washing a root of a *Cryptolepis* sp. with an alcoholic solvent to obtain an alkaloid salt-containing alcoholic solution; and

25 (b) concentrating the alkaloid salt-containing alcoholic solution to obtain an alkaloid salt-containing residue useful as a hypoglycemic agent.

30 15. The method according to claim 14, in which the extract was obtained by a process which further comprised:

(c) diluting the alkaloid salt-containing residue with water to obtain an alkaloid salt-containing aqueous solution;

35 (d) washing the alkaloid salt-containing aqueous solution with a non-polar organic solvent to remove neutral extractables therefrom;

() washing the alkaloid salt-containing aqueous solution of step (d) with base to obtain a free base-containing aqueous solution;

5 (f) extracting the free base-containing aqueous solution with a non-polar organic solvent to obtain a free base-containing organic solution; and

(g) concentrating the free base-containing organic solution to obtain a free base-containing extract.

10 16. The method according to claim 14 in which the *Cryptolepis* sp. is selected from *C. triangularis* and *C. sanguinolenta*.

15 17. The method according to claim 15, in which the extract further comprises a pharmaceutically acceptable carrier.

20 18. A method for reducing the blood glucose of a mammal, comprising administering to a mammal in need of such blood glucose reduction, an effective amount of an extract from a *Cryptolepis* sp. in which said extract was obtained by a process which comprised:

(a) washing a root of *Cryptolepis* sp. with an organic solvent to obtain an alkaloid salt-containing marc, 25 said alkaloid salt-containing marc being free of neutral extractables;

(b) wetting the alkaloid salt-containing marc with base to obtain a free base-containing marc;

30 (c) washing the free base-containing marc with an alcoholic solvent to obtain a free base-containing alcoholic solution; and

(d) concentrating the free base-containing alcoholic solution to obtain a free base-containing extract.

35 19. A method for treatment of diabetes mellitus, comprising administering, to a mammal suffering from diabetes mellitus a therapeutically active amount of an extract from a

cryptolepis sp., in which said extract was obtained by a process which comprised:

- (a) washing a root of *Cryptolepis* sp. with an organic solvent to obtain an alkaloid salt-containing marc, said alkaloid salt-containing marc being free of neutral extractables;
- (b) wetting the alkaloid salt-containing marc with base to obtain a free base-containing marc;
- (c) washing the free base-containing marc with an alcoholic solvent to obtain a free base-containing alcoholic solution; and
- (d) concentrating the free base-containing alcoholic solution to obtain a free base-containing extract.

15 20. The method according to claim 19, in which the method for obtaining the extract further comprised: (e) purifying the free base-containing residue to obtain a purified extract.

20 21. The method according to claim 19, in which the *Cryptolepis* sp. is selected from *C. triangularis* and *C. sanguinolenta*.

22. The method according to claim 19, in which the extract further comprises a pharmaceutically acceptable carrier.

25 23. The method according to claim 22, in which the pharmaceutically acceptable carrier is water or an aqueous solution.

30 24. The method according to claim 19, in which the extract is administered in conjunction with another hypoglycemic agent selected from the group consisting of a 35 sulfonylurea, a biguanide, a thiazolidinedione, a β -adrenoceptor agonist, an α -glucosidase inhibitor and insulin.

25. A pharmaceutical composition for use as a hypoglycemic agent in mammals, comprising a therapeutically effective amount of an extract of a *Cryptolepis* sp. obtained by a method comprising:

5 (a) washing a root of a *Cryptolepis* sp. with an alcoholic solvent to obtain an alkaloid salt-containing alcoholic solution; and

10 (b) concentrating the alkaloid salt-containing alcoholic solution to obtain an alkaloid salt-containing residue.

26. The composition according to claim 25, in which the extract was obtained by a process which further comprised:

15 (c) diluting the alkaloid salt-containing residue with water to obtain an alkaloid salt-containing aqueous solution;

20 (d) washing the alkaloid salt-containing aqueous solution with a non-polar organic solvent to remove neutral extractables therefrom;

25 (e) washing the alkaloid salt-containing aqueous solution of step (d) with base to obtain a free base-containing aqueous solution;

(f) extracting the free base-containing aqueous solution with a non-polar organic solvent to obtain a free base-containing organic solution; and

30 (g) concentrating the free base-containing organic solution to obtain a free base-containing residue to obtain an extract suited for use as a hypoglycemic agent.

27. The composition according to claim 26, in which the extract was obtained by a method which further comprises:

35 (h) purifying the free base-containing residue by means of HPLC to obtain a purified extract suited for use as a hypoglycemic agent.

28. A pharmaceutical composition for use as a hypoglycemic agent in mammals, comprising a therapeutically

effective amount of an extract of *Cryptolepis* sp. obtained by a method comprising:

(a) washing a root of a *Cryptolepis* sp. with an organic solvent to obtain an alkaloid salt-containing marc, said alkaloid salt-containing marc being free of neutral extractables;

(b) wetting the alkaloid salt-containing marc with base to obtain a free base-containing marc;

(c) washing the free base-containing marc with an alcoholic solvent to obtain a free base-containing alcoholic solution;

(d) concentrating the free base-containing alcoholic solution to obtain a free base-containing residue to obtain an extract suited for use as a hypoglycemic agent.

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29. The composition according to claim 28, in which the extract was obtained by a method which further comprises:

(e) purifying the free base-containing residue to obtain a purified extract suited for use as a hypoglycemic agent.

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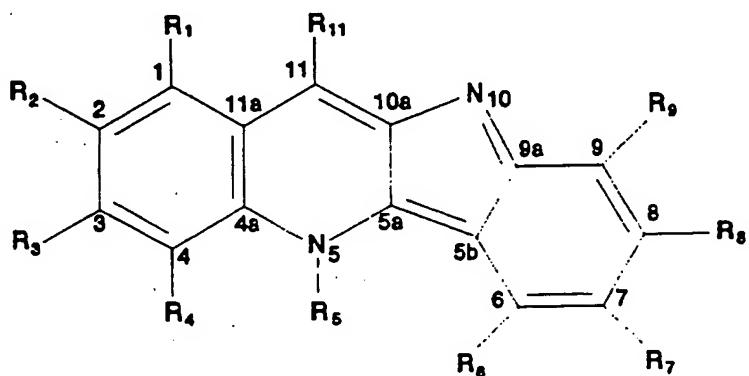
30. A method for reducing the blood glucose of a mammal, comprising administering to a mammal in need of such blood glucose reduction an effective amount of an extract from a *Cryptolepis* sp., in which said extract was obtained by a process which comprises: steeping crushed root of a *Cryptolepis* sp. in water or an aqueous solution at about 25 - 100°C, removing the residual solid root material, and drying the remaining aqueous solution to obtain said extract.

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31. A method for treating diabetes mellitus, comprising administering to a mammal in need of such treatment, a therapeutically effective amount of an extract of a *Cryptolepis* sp., in which said extract was obtained by a process which comprises: steeping crushed root of a *Cryptolepis* sp. in water or an aqueous solution at about 25 -

100°C, removing the residual solid root material and drying the remaining aqueous solution to obtain said extract.

32. A method for lowering the blood triglyceride
5 level of a mammal, comprising administering to a mammal in
need of such triglyceride reduction, an effective amount of a
composition comprising a compound having the following
structure or a pharmaceutically acceptable salt thereof



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said compound selected from group consisting of:

25 (a) where $R_1 - R_{11} = H$;
 (b) where $R_1 - R_4$ and $R_6 - R_{11} = H$, and $R_5 = CH_3$;
 (c) where $R_1 - R_4$ and $R_6 - R_{11} = H$, and R_5 is selected from the group of ethyl, isopropyl, benzyl, 4-hydroxybenzyl, 4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl, 3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl, 3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, 4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino, 2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-

fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-imidazolylmethyl, 4-imidazolylmethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;

5 (d) where $R_1, R_4 R_6 - R_{11} = H$, $R_2 = R_3 = -CH_2-O-CH_2-$,
 and R_5 is selected from the group consisting of ethyl,
 isopropyl, benzyl, 4-hydroxybenzyl, 4-dimethylaminobenzyl,
 4-chlorobenzyl, phenyl, 2-chlorophenyl, 3-chlorophenyl, 2-
 bromophenyl, 3-bromophenyl, 2-fluorophenyl, 3-fluorophenyl, 2-
 10 iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-
 hydroxyphenyl, 2-dimethylaminophenyl, 3-dimethylaminophenyl,
 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl,
 4-hydroxyphenyl, 4-dimethylaminophenyl, 4-chlorophenyl,
 2-pyridino, 3-pyridino, 4-pyridino, 2-imidazol, 4-imidazol, 2-
 15 hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-
 dimethylaminobenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-
 methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl,
 3-bromobenzyl, 4-bromobenzyl, 2-fluorobenzyl, 3-fluorobenzyl,
 4-fluorobenzyl, 2-iodobenzyl, 3-iodobenzyl, 4-iodobenzyl, 2-
 20 pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-
 imidazolylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-
 fluorophenyl, cyclopropyl, and isobutyl;

(e) where $R_1 = R_4$, $R_6 = R_7$ and $R_{10} = R_{11} = H$, $R_8 = R_9 = -CH_2-O-CH_2-$, and R_5 is selected from the group consisting of
25 ethyl, isopropyl, benzyl, 4-hydroxybenzyl,
4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl,
3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl,
3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-
hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-
30 dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-
methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl,
4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino,
2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-
dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl,
35 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-
chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-
fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-

iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;

(f) where $R_1 - R_3$, $R_6 - R_{11} = H$, and $R_4 - R_5 =$

5 $-\text{CH}_2\text{CH}_2-$;

(g) where $R_1 - R_3$, $R_6 - R_{11} = H$, and $R_4 - R_5 =$

$-\text{CH}_2\text{CH}_2\text{CH}_2-$;

(h) where $R_1 - R_4$ and $R_6 - R_{11} = H$, $R_5 = \text{CH}_3$, and 10a, 11 = dihydro;

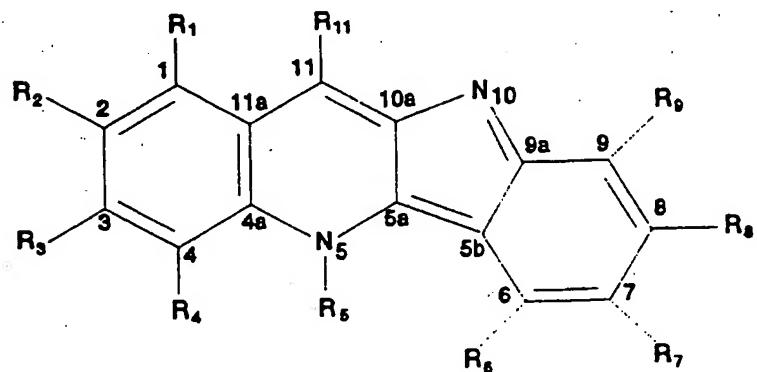
10 (i) where $R_1 - R_4$ and $R_6 - R_{11} = H$, $R_5 = \text{CH}_3$, and 5a, 5b = dihydro;

(j) where $R_1 - R_4$ and $R_6 - R_{11} = H$, $R_5 = \text{CH}_3$, and 5a, 5b, 10a, 11 are tetrahydro;

(k) where $R_1 - R_{11} = H$, and 9a, 10 = dihydro; and

15 (l) where $R_1 - R_{11} = H$, and 10-methyl.

33. A compound having the formula:



I

30 said compound selected from group consisting of:

(a) where $R_1 - R_4$ and $R_6 - R_{11} = H$, and R_5 is selected from the group of ethyl, isopropyl, benzyl, 4-hydroxybenzyl, 4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl, 3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl, 3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-

dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, 4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino, 2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;

(b) where $R_1, R_4 R_6 - R_{11} = H$, $R_2 = R_3 = -CH_2-O-CH_2-$, and R_5 is selected from the group consisting of ethyl, isopropyl, benzyl, 4-hydroxybenzyl, 4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl, 3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl, 3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, 4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino, 2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;

(c) where $R_1 - R_4, R_6 - R_7$ and $R_{10} - R_{11} = H$, $R_8 = R_9 = -CH_2-O-CH_2-$, and R_5 is selected from the group consisting of ethyl, isopropyl, benzyl, 4-hydroxybenzyl, 4-dimethylaminobenzyl, 4-chlorobenzyl, phenyl, 2-chlorophenyl, 3-chlorophenyl, 2-bromophenyl, 3-bromophenyl, 2-fluorophenyl, 3-fluorophenyl, 2-iodophenyl, 3-iodophenyl, 4-iodophenyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 2-dimethylaminophenyl, 3-dimethylaminophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, 4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino, 2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl, 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;

methoxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl,
4-chlorophenyl, 2-pyridino, 3-pyridino, 4-pyridino,
2-imidazol, 4-imidazol, 2-hydroxybenzyl, 3-hydroxybenzyl, 2-
dimethylaminobenzyl, 3-dimethylaminobenzyl, 2-methoxybenzyl,
5 3-methoxybenzyl, 4-methoxybenzyl, 2-chlorobenzyl, 3-
chlorobenzyl, 2-bromobenzyl, 3-bromobenzyl, 4-bromobenzyl, 2-
fluorobenzyl, 3-fluorobenzyl, 4-fluorobenzyl, 2-iodobenzyl, 3-
iodobenzyl, 4-iodobenzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-
pyridylmethyl, 2-imidazolylmethyl, 4-imidazolymethyl, 4-
10 bromophenyl, 4-fluorophenyl, cyclopropyl, and isobutyl;
(d) where $R_1 = R_3$, $R_6 = R_{11} = H$, and $R_4 = R_5 =$
-CH₂CH₂-;
(e) where $R_1 = R_3$, $R_6 = R_{11} = H$, and $R_4 = R_5 =$
-CH₂CH₂CH₂-;
15 (f) where $R_1 = R_4$ and $R_6 = R_{11} = H$, $R_5 = CH_3$, and
10a, 11 = dihydro;
(g) where $R_1 = R_4$ and $R_6 = R_{11} = H$, $R_5 = CH_3$, and 5a,
5b = dihydro;
(h) where $R_1 = R_4$ and $R_6 = R_{11} = H$, $R_5 = CH_3$, and 5a,
20 5b, 10a, 11 = tetrahydro;
(i) where $R_1 = R_{11} = H$, and 9a, 10 = dihydro; and
(j) where $R_1 = R_{11} = H$, and 10-methyl.

FIG. 1

Isolation of Cryptolepine from *Cryptolepis sanguinolenta* roots

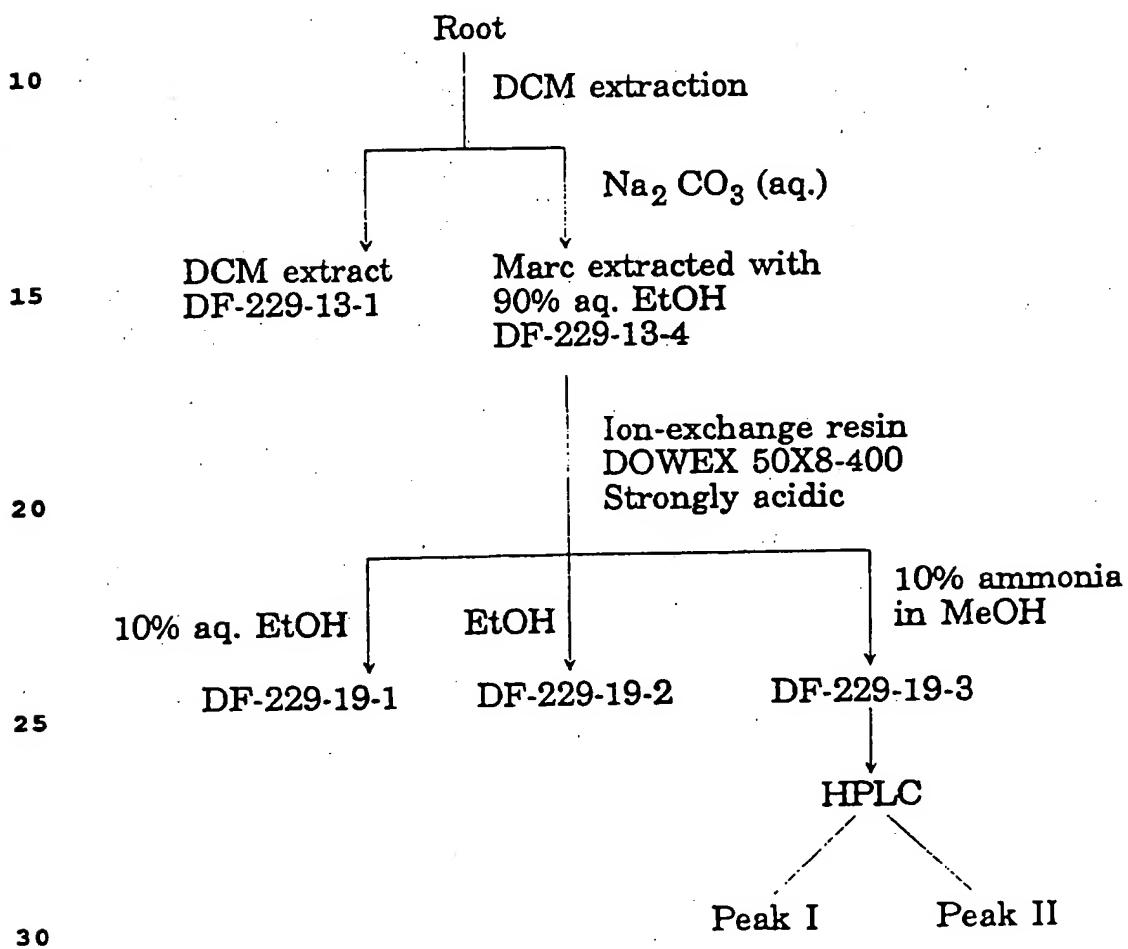


FIG. 2

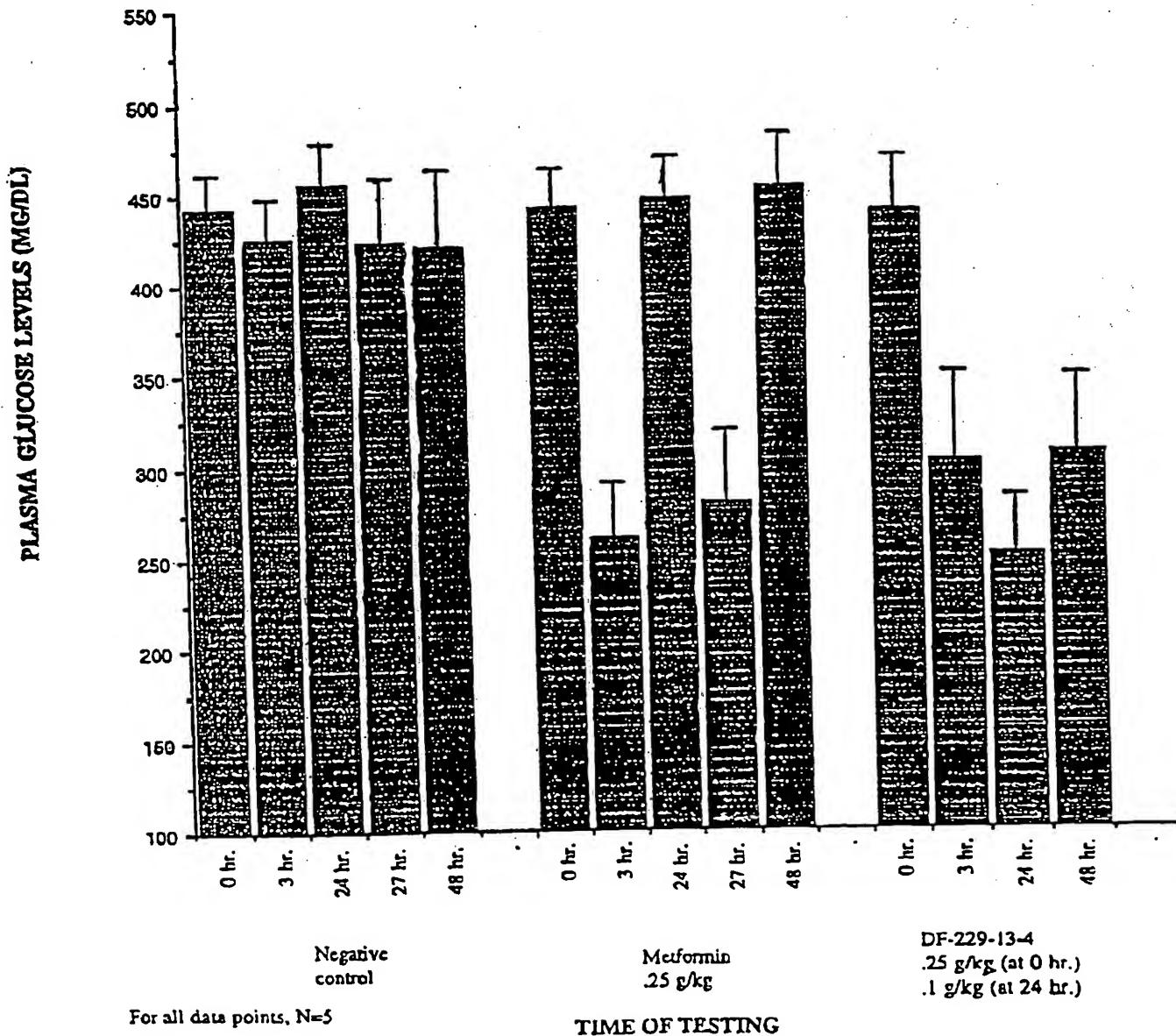
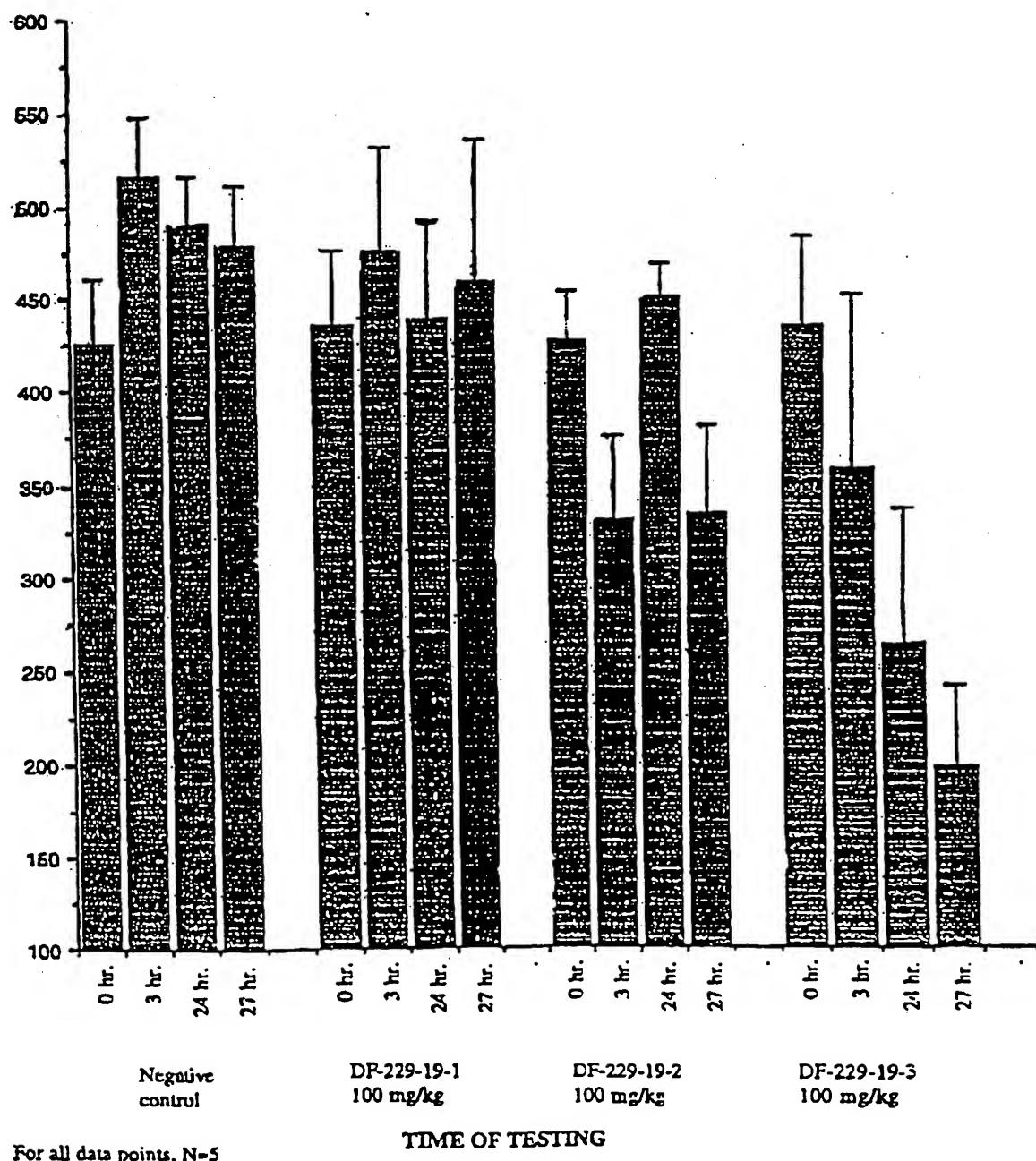
ANTI-HYPERGLYCEMIC ACTIVITY
OF DF-229-13-4

FIG. 3

ANTI-HYPERGLYCEMIC ACTIVITY
OF DF-229-19-3

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FIG. 4

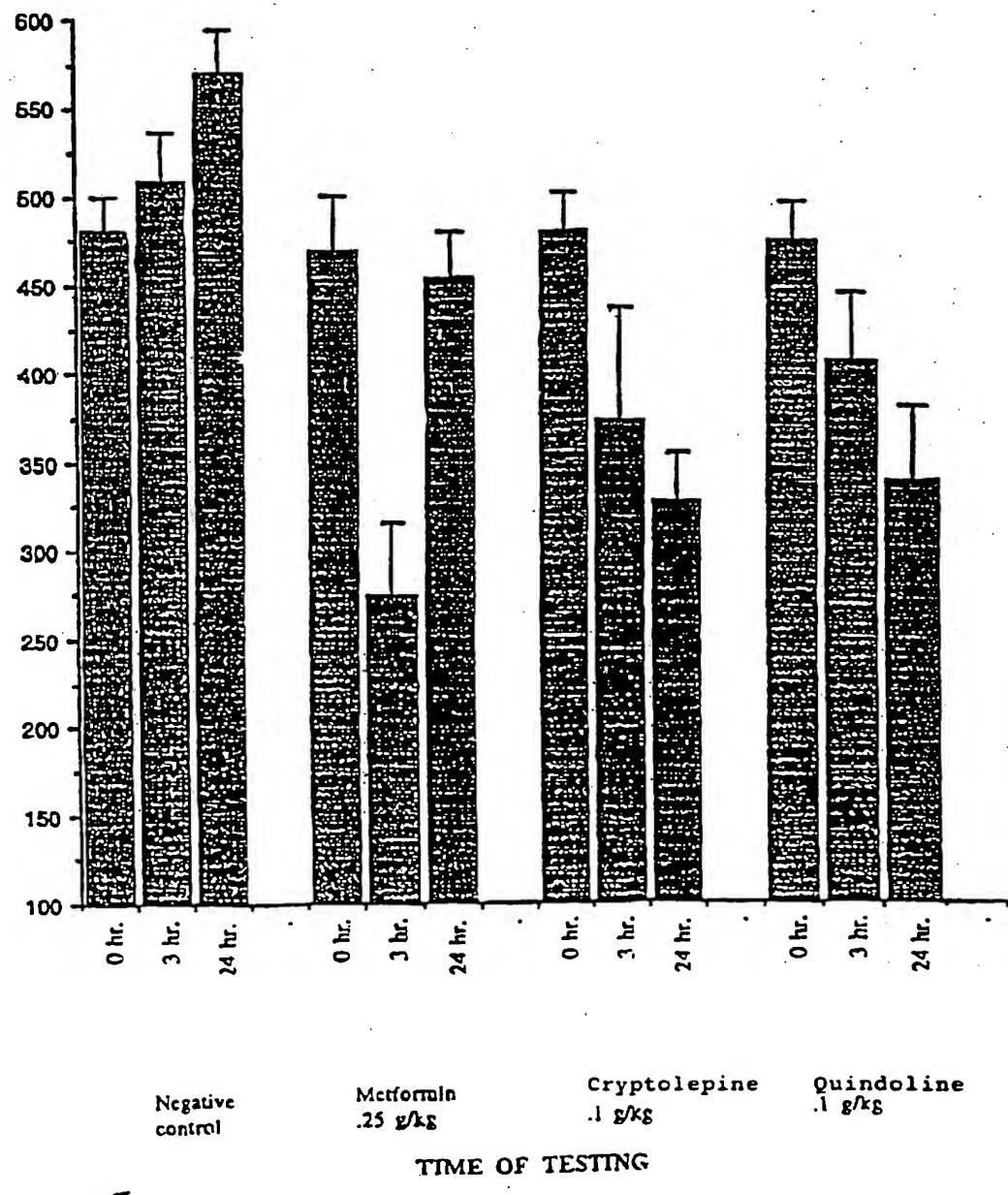
ANTI-HYPERGLYCEMIC ACTIVITY
OF CRYPTOLEPINE AND QUINDOLINE

FIG. 5A

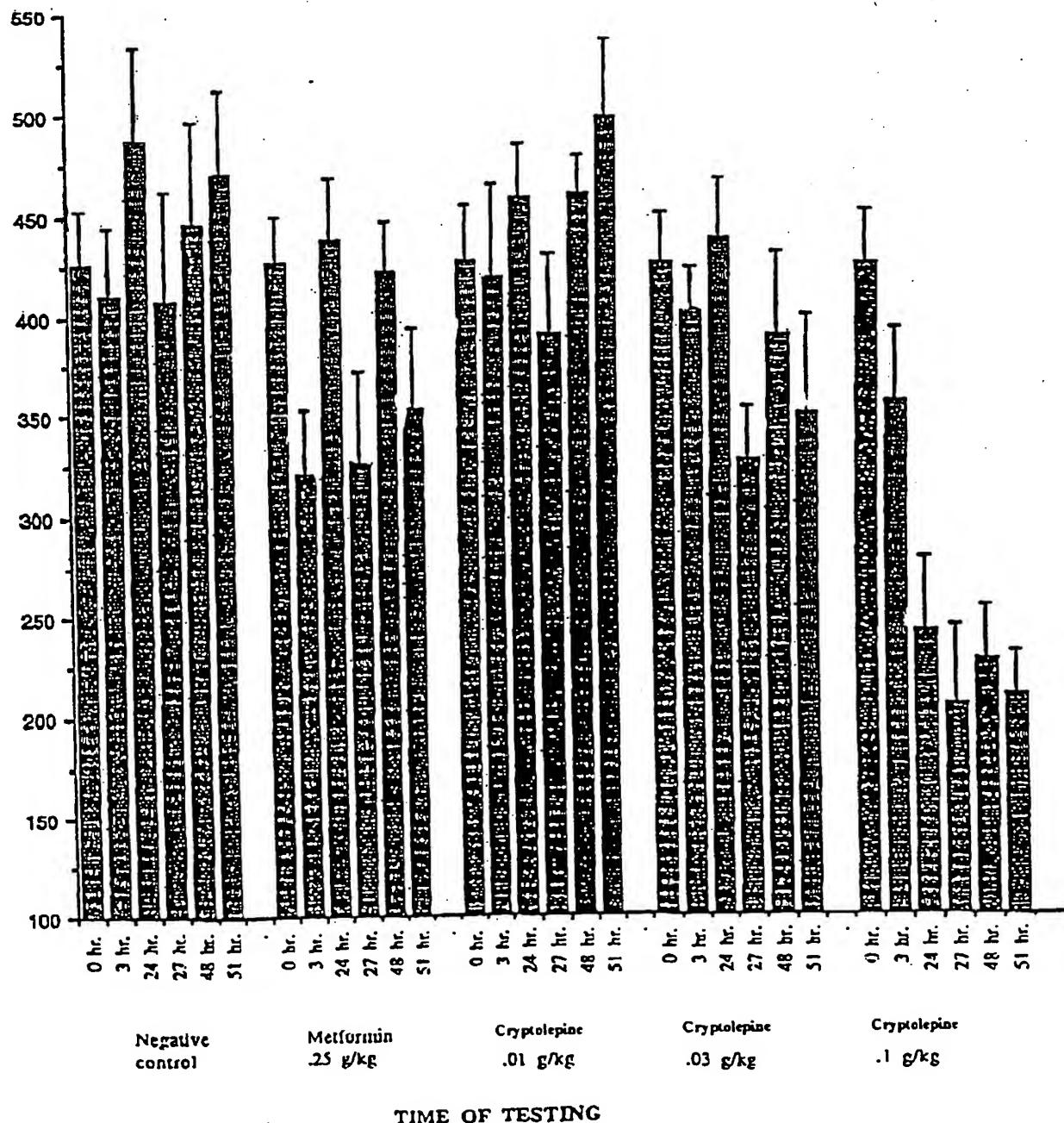
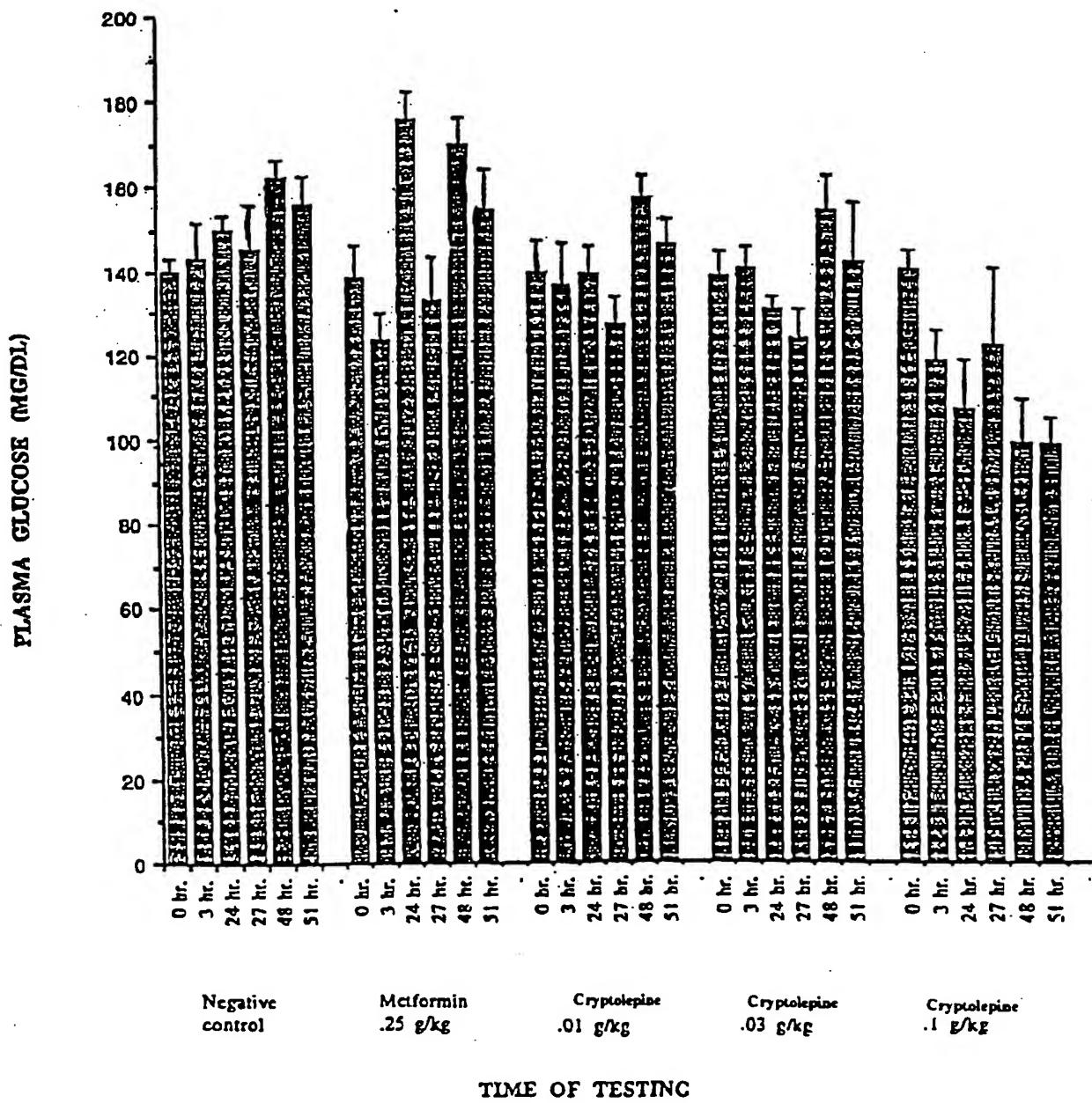
ANTI-HYPERGLYCEMIC ACTIVITY OF
CRYPTOLEPINE IN DIABETIC MICE

FIG. 5B

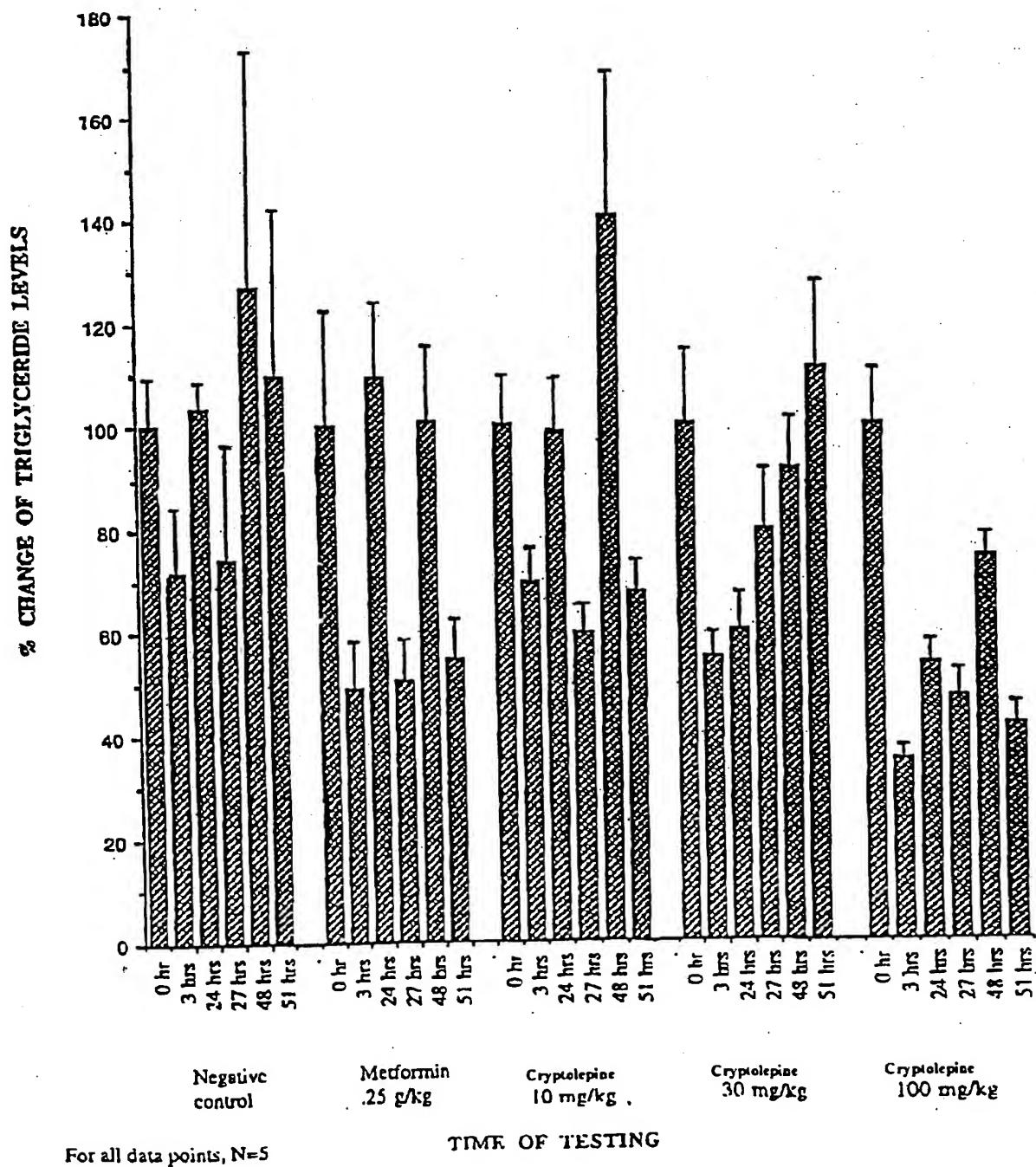
**EFFECTS OF CRYPTOLEPINE ON
PLASMA GLUCOSE LEVELS IN LEAN MICE**



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FIG. 6A

EFFECTS OF CRYPTOLEPINE ON PLASMA TRIGLYCERIDE LEVELS IN DIABETIC MICE



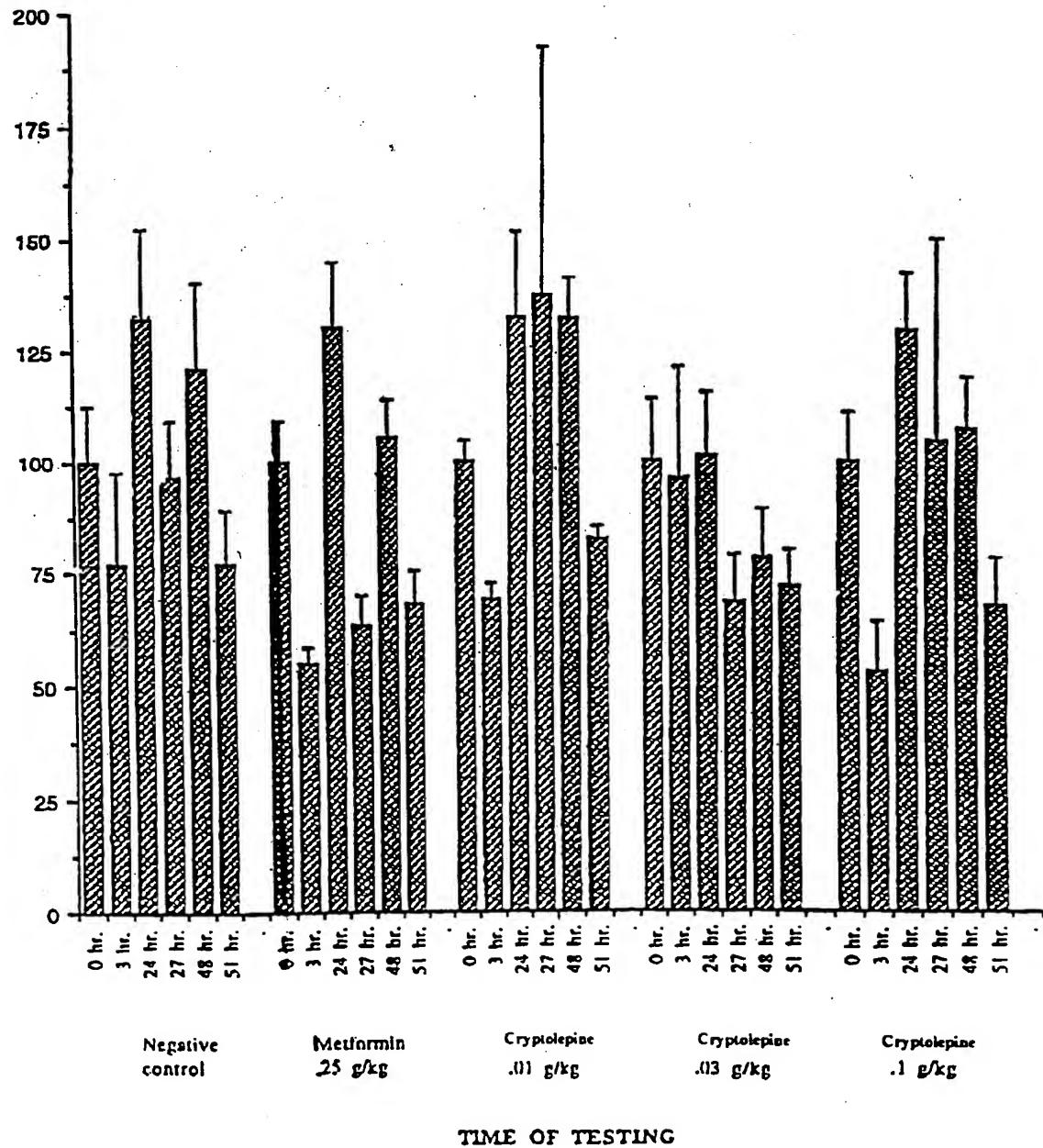
For all data points, N=5

TIME OF TESTING

FIG. 6B

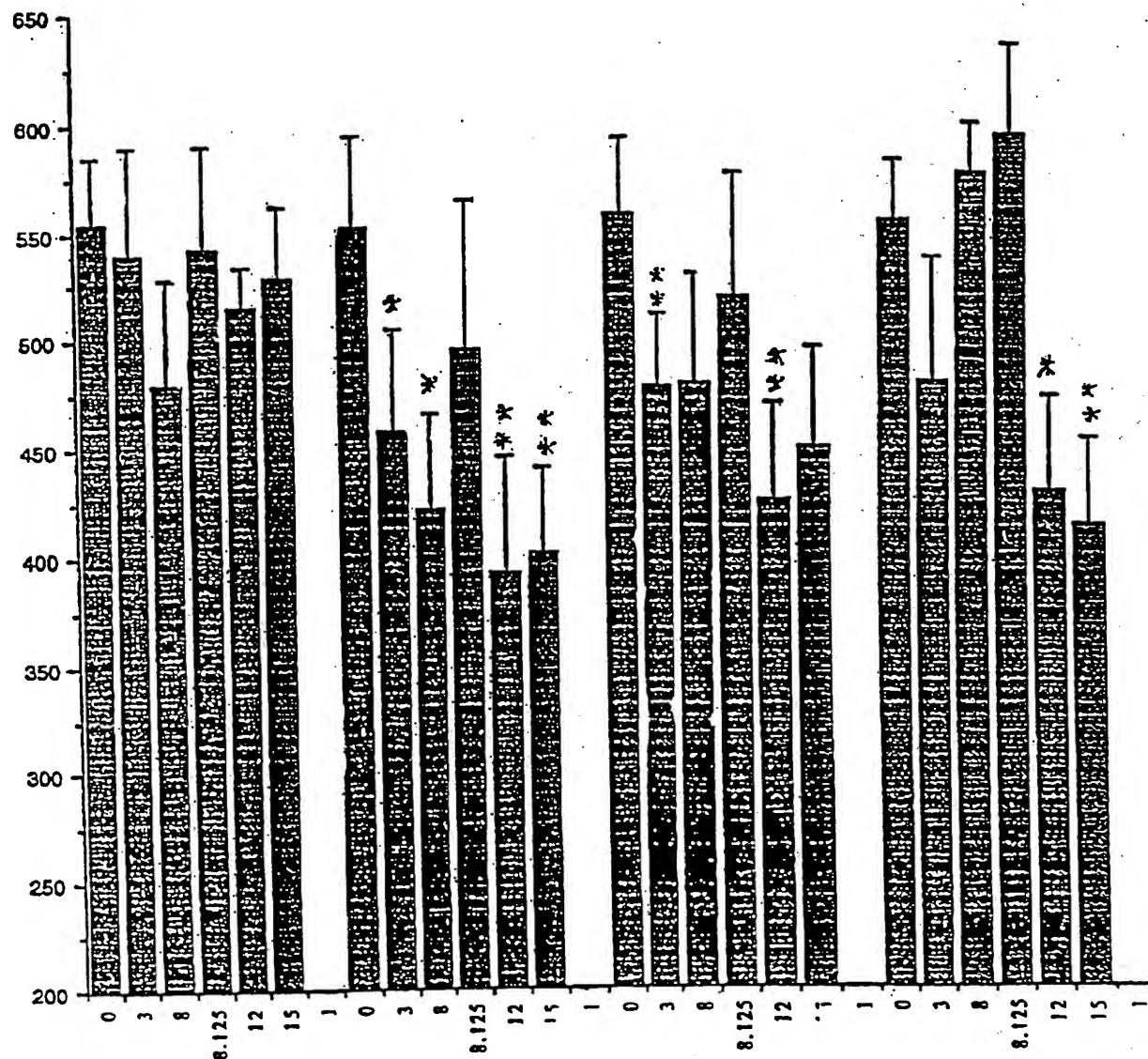
EFFECTS OF CRYPTOLEPINE ON PLASMA TRIGLYCERIDE LEVELS IN LEAN MICE

% CHANGE OF TG LEVELS



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FIG. 7

ANTI-HYPERGLYCEMIC ACTIVITY OF
AQUEOUS EXTRACT IN DIABETIC MICE

N-con

Metformin
.25 g/kgAqueous Extract
.25 g/kgAqueous Extract
1.0 g/kg

For all data points, N=8. Student paired t test was used to compare pre- and post-dosing glucose levels

* P < 0.05

** P < 0.01

DAYS OF TESTING (DAY)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/12505

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/42, 31/40, 35/78; C07D 209/80
US CL :514/285, 410, 422, 867, 884; 424/195.1; 548/420

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/285, 410, 422, 867, 884; 424/195.1; 548/420

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Journal of Cardiovascular Pharm., Volume 23, issued 1994, A. Oyekan, "Role of the Endothelium and Cyclic GMP in Renal Vasodilator Responses to Cryptolepine in Rats", pages 602-611, see entire document.	1-33
A	Planta Medica, Volume 58, issued 1992, Rauwald, et al., "Cryptolepis Sanguinolenta: Antimuscarinic Properties of Cryptolepine and the Alkaloid Fraction at M1, M2 and M3 Receptors", pages 486-488, see entire document.	1-33

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	
A document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
E earlier document published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
20 DECEMBER 1995

Date of mailing of the international search report

11 JAN 1996

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